PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Upon the Existence of More than One Fungus in Madura Disease (Mycetoma)

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PHILOSOPHICAL TRANSACTIONS.

I. *Upon the Existence of more than one Fungus in Madura Disease (Mycetoma).*

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[Plates 1–4.]

The scientific history of the fungus disease of India dates from the publication, in 1874, of the collected papers of Vandyke Carter. This observer showed that the fungus foot was a "veritable parasitic disease, due to the growth and extension within the tissues of the human foot of an indigenous mould." He demonstrated the presence of the parasite in all specimens examined, and came to the conclusion that it was one species, the *Chionyphe Carteri.*

In 1888 new observations were published in the collected papers of Lewis and Cunningham. They came to the conclusion that mycetoma was "essentially a degeneration of the fatty tissues, independent of the local presence or influence of any parasites whatever."

In 1888, Bassini* described a case of the black variety of the fungus in Italy (the first case in Europe), and by means of caustic potash concluded that the organism was a mycelial fungus, somewhat of the nature of the *Aspergilli* or *Mucorini.*

Lastly, in 1892, Dr. Kanthack† re-asserted the fungoid nature of the disease, and brought forward evidence to show the identity, or close affinity, of the organism with that of *Actinomycosis.* We, on the other hand, will bring forward reasons for believing that there are at least two distinct fungi—one a very highly organised species, the other a very delicate and lowly organised type, presenting very many of

* Bassini, 'Bact. Centrlb.,' 1888.

† 'Journal of Pathology and Bacteriology,' No. 2.

the characters of *Actinomyces*. And we further think that we will be able to account for the apparent discrepancies in observation in the case of the previous writers.

The Madura foot of India is a very chronic affection, lasting in some cases twenty-five years.* It is a purely local disease of the extremities, chiefly the foot, and generalisation has not been observed. It usually occurs in people who go barefoot and are working in fields. In most cases it has been traced to some injury. The big toe is often affected at first. The disease stops at the ankle for a short time, then it spreads up to the knee, and eventually may even reach the thigh. One of us has had the opportunity of seeing a recurrence in the scar after amputation; this, however, must be very rare, and amputation affords complete relief—and is, indeed, one of the most successful operations in India. The foot, as the photograph 1 shows, is greatly altered; it is enlarged, often many times the natural size. The overgrowth of the foot is irregular; the toes may become buried, as shown in the figure, and the surface become studded over with mammillated, or even villous, projections. A large number of the mammillated projections mark the presence of sinuses, which pass deeply into the foot; and on section (fig. 2) these may be seen to honeycomb it. From the opening of the sinuses a purulent or sanious discharge can be pressed out, and in this are found, in one series of cases, small particles of a light yellow colour, which have been compared to fish roe; whilst, in the remaining cases, deep brown or black particles, resembling grains of gunpowder, may be seen. The disorganisation of the interior of the foot becomes very complete in time; the bones undergo a rarefactive osteitis, and are ultimately absorbed; granulation tissue also invades the muscles and fat, and leads to their disappearance. Associated with the hypertrophy of the granulation tissue there may be considerable hyperplasia of the epithelium of the skin. This overgrowth gives rise to the mammillated and papillomatous projections previously referred to.

The only difference which one at present finds, clinically and microscopically, in the numerous cases of Madura foot is the difference in the size and colour of the particles. CARTER termed that form of the disease in which the black particles were present the "melanoid" variety, while under the "white" or "ochroid" were grouped those cases in which the fish roe-like bodies were found. It will be understood that only those cases of Madura disease in which the *particles* can be demonstrated macroscopically or microscopically will be admitted as genuine. The disease appears to be confounded with scrofula and various forms of elephantiasis, in the production of which probably other parasites play an important part. In this paper we will confine ourselves chiefly to the elucidation of the fungus of the melanoid variety, and contrast it with that of the white. This is all the more necessary, because the older writers, from the time of VANDYKE CARTER, agreed that the black bodies did show some kind of vegetable structure, whilst the structure of the white appeared very obscure. Yet the most recent writer upon the subject, Dr. KANTHACK, takes the opposite view,

* NUSSERWANGI F. SURVEYOR, "Madura Foot of India." 'Brit. Med. Journ.,' Sept., 1892.

and in the three melanoid specimens which he examined he "found it difficult to convince himself of their vegetable nature."

On examining a diseased foot, the black particles will be found in abundance, both loose in the sinuses and packed closely in cavities in the tissue. A fresh section with the razor may cut across a black mass firmly imbedded in the tissue, and the intimate relationship of both can then be readily seen with the naked eye. The particles vary very greatly in size, from a pin's head, or even smaller, to the large mass three-quarters of an inch in diameter, depicted in fig. 3. In shape they are irregular, and a dendritic form can very often be seen. Fig. 3 has the mulberry form which is often described. On section, the appearance is somewhat radiate. Fig. 4 is a section through the large mass (fig. 3), slightly magnified (Obj. 35 mm., ZEISS). Fig. 5 passes through a minute particle in another specimen; it is more highly magnified (Obj. D, ZEISS), and the appearance is distinctly radiate, but this fine radiation is not the same as the coarse radiate grouping seen with the naked eye, and in the preceding figure. As regards colour, the naked eye examination shows that the particles are darkest at the periphery, becoming a lighter brown as the centre is approached. Examinations with the immersion, as in fig. 6, seem at first sight to throw very little light upon the nature of these curious black masses; a slight venation may be seen, as in fig. 6, or peculiar holes in a homogeneous matrix, as in figs. 5 and 6A. It was in these apparently structureless masses that we first demonstrated the existence of a well-formed, large-branching, and septate fungus. The method of procedure was as follows:—A black particle was boiled for from one minute to half an hour in concentrated caustic potash; the particle, which had undergone very little apparent change, was next transferred to distilled water, when all the black colouring matter rapidly diffused out, leaving behind a white soft mass. This, upon microscopic examination, proved to consist wholly of a fungus similar to that represented in figs. 7–9. A segment of the large black mass (fig. 3) was, after similar treatment with caustic potash and water, dehydrated and imbedded in collodion; sections subsequently revealed a similar fungus. Caustic potash, we found, had been used by other observers. Thus, LEWIS and CUNNINGHAM direct that a crushed particle should be placed for a considerable time in a test-tube of strong caustic potash; a sediment collects at the bottom which equals the $\frac{1}{50}$th of the original bulk; upon microscopic examination, this proves to consist of hyphæ not distinguishable from those of fungi. From an illustration of the hyphæ given by the authors, it appears they are identical with those we find. They, however, look upon these elements as unimportant constituents of the black masses, which they think have an origin identical with the other pigmentary deposits in animal tissues. The fungus is to them a mere "epiphenomenon" in the particle. In our method of treatment with boiling caustic potash and water, we in no way disturb the arrangement of the fungus, and we have succeeded in washing and mounting sections of particles as small as a pin's head. The method, however, has the disadvantage that it did not permit us to examine the decolourized fungus *in situ*

in the tissues, any trace of animal tissue being destroyed by the concentrated caustic potash, nor does it permit the use of collodion. We had at this stage to content ourselves with the examination of the brown amorphous masses in the tissues, and of the decolourized particles after removal from them.

Professor OLIVER,* however, brought to our notice the use of *Eau de Javelle* as a clearing re-agent superior to caustic potash for vegetable structure. Its application in our case was most successful. If the black particles are dehydrated, embedded in collodion, and cut, and the sections are then steeped in the *Eau de Javelle* for from two to five minutes, or until gas bubbles begin to appear in them, it will be found that the fungus has been rendered beautifully transparent, whilst remains of the original animal tissue are still visible. In fact, within certain limits this re-agent has very little action upon the animal tissue, and thus at once permits the study of the clarified fungus in relation to its surroundings. It suffices for this purpose that a portion of the tissue containing the black masses should be thoroughly dehydrated in absolute alcohol, then transferred to ether, and subsequently to collodion. The collodion is allowed to concentrate, and the impregnated mass is hardened in methylated chloroform, and cut. The sections are washed in the *Eau de Javelle* until almost all the colour has disappeared from the particles; they are then rinsed in plenty of distilled water and stained with logwood or hæmatoxylin. No matter how old the specimen, and we have examined the oldest in this country, the results are always the same.

The following are the results obtained from an examination of seven specimens of the black variety.†

Fig. 4 is a very slightly magnified section of the large mass, fig. 3, obtained from the specimen fig. 2 in University College Museum. It will be seen that it is composed of irregular black masses, which, more especially towards the periphery, have a somewhat branched or radiate disposition. Fig. 6 is portion of one of the dark masses in the preceding figure, seen under the $\frac{1}{12}$ immersion; it shows the indication of a branching network, the branches for the most part running fan-like to the periphery; there is a peculiar oval body in the lower part of the section. Examination of the untreated sections showed the presence of very many of these oval bodies, but some were more round and others more oval in section; during our early experiments with caustic potash their appearance greatly puzzled us. It had occurred to us that they might be sections of vessels, but we could hardly realize how vessels could have become imbedded in the brown amorphous material. Professors MARSHALL WARD and OLIVER likewise

* We wish to express our thanks both to Professor OLIVER and Professor MARSHALL WARD for much friendly criticism and advice during the course of this investigation.

† We are greatly indebted to Mr. CATHCART for specimens from the Museum of the College of Surgeons in Edinburgh; to Mr. TARGETT, of the Royal College of Surgeons, London; to Dr. WILLETT, of St. Bartholomew's; to Dr. LAWRENCE of the University College Museum; to Professor WRIGHT, of Netley, and Professor COATES, of Glasgow; to Dr. BENNETT, of Trinity College, Dublin, and to Dr. SHAW, of Guy's Hospital, and Dr. MASSINA, of Bombay. See end of paper for notes respecting fifteen other cases examined since this paper was written.

suggested that they might be vessels, and such we consequently found most of them to be. Fig. 10 is a photomicrograph, taken with Obj. BB., Zeiss, of portion of the section fig. 4 which has been treated with *Eau de Javelle* and stained for twenty-four hours in logwood. In it the lighter areas correspond to the slightly stained fungus, whilst the dark network indicates the remains of the animal tissue in which the fungus grew; the bodies conspicuous amongst the hyphal masses are for the most part *vessels*. When fig. 10 is examined with the immersion, the various vegetable and animal elements can be readily made out. Figs. 7 and 8, taken from decolourized unstained glycerine preparations, show that the hyphæ are large, irregularly branched, and septate, and their appearance seems more suggestive of the higher *sprouting fungi* than of any other group with which we are acquainted. The grouping, size, and division of the filaments is, however, subject to considerable variation. The fungus is most frequently met with in the tissue in the form of *tufts*, made up of hyphæ which spread out somewhat fan-like; in a less number of cases the filaments in a tuft radiate regularly from the centre; in a third case (figs. 11 and 12) the periphery is made up of closely-set radiating hyphæ, whilst the centre is occupied by an irregular large-celled pseudo-parenchyma. It was a specimen of this last form that we are inclined to believe Dr. Kanthack mistook for *Actinomyces*, and upon which he grounded the opinion of the Actinomycotic nature of the white and black varieties of Madura foot. The hyphæ may be very slender, with few septa and branches, a condition which is mostly seen in what appear to be the rapidly growing forms. There may be marked segmentation and branching, the segments may be very large and oval (fig. 8), and in some cases they give rise to a very striking pseudo-parenchyma. Very often one segment is very much larger than the others, and it may be terminal and then resemble somewhat closely a very large spore capsule. In the radiate tufts the hyphæ usually taper towards the periphery. Many of the hyphæ and large cell segments contain granular contents, in spite of the *Eau de Javelle* treatment. The walls of the hyphæ vary very considerably in thickness; sometimes they are as thick as the lumen is wide, and they then cause the filaments in transverse sections to stand out pipe-like; the walls also of the large rounded segments are often irregularly and very greatly thickened; occasionally the walls of the terminal segments of a tuft are thickened, in a club-like manner, fig. 17A. In one case of the disease the majority of the hyphæ were comparatively delicate, fig. 9; in another very large and round or oval (fig. 8). The hyphæ may be very closely packed together into a feltwork, or into a large-celled pseudo-parenchyma, in which the walls of the segments appear to have fused together. On the other hand, the filaments may be widely separated from one another, the intermediate substance being a finely granular ground-glass-like material. This latter substance possesses in the untreated specimens the beautiful golden brown colour which is very characteristic (fig. 6A). The filaments in the centre of the tufts may undergo necrosis. Apart from the colouring material which impregnates the intermediate substance and the walls of the hyphæ, and which can be readily removed

by *Eau de Javelle,* there may exist a much more persistent brown pigmentation of the walls of the filaments (fig. 17A). In no specimen have we seen any indication of the formation of sporangia or of spores.

With regard to the staining reactions of the fungus, we find that logwood, as in the case of the hyphal fungi, generally gives the best results; we further find that the use of the *Eau de Javelle increases* the staining power of the logwood. The filaments often stain unequally; the terminal segments, as a rule, stain more deeply than the others.

The Nature of the Tissue Changes produced by the Growth of the Fungus.

The macroscopic appearances of the foot show that the parasite produces wide-spread hyperplasia as well as tissue destruction. When the relationship of the black particles to the surrounding tissue is more closely studied, it is seen, as previously stated, that the former lie either loosely grouped in the sinuses, or closely surrounded by the tissues and semi-encapsuled. The large body (fig. 3) was encapsuled, but could, nevertheless, be readily enucleated. The readiness with which this mass was removed, as well as its compact nature and appearance on section (fig. 4), led us at first to suppose that it would contain no traces of animal tissue; the clearing away of the yellow colouring material and prolonged staining, however, clearly demonstrated the remains of the tissue in which the fungus grew. The network formed by this tissue is seen in fig. 10, as well as the skeletons of the vessels to which we have already called attention. Fig. 13 is portion of a trabecula of fig. 10, seen under the immersion, and it shows the branching hyphæ dipping into granulation tissue. The drawing (fig. 19) shows the same thing as well as the gradual loss of distinctness of the small round cells; many of the trabeculæ are simply represented by tissue which has undergone coagulation necrosis, and in which, consequently, it is hard to demonstrate any cell elements. Fig. 5, the photograph, and fig. 6A, the drawing, of a radiating tuft of the uncleared fungus, show the massing of small round cells in the immediate vicinity of the fungus. Both in the photograph and in the drawing, the gradual passage of the infiltrated tissue into the homogeneous ground-glass-like and pigmented interstitial substance between the hyphæ of the fungus is very striking. In all preparations of Madura disease, the round celled massing is very obvious, but in some cases the progressive destruction of the infiltrated tissue by the fungus is much more obvious than in other cases. Thus, instead of the fungus being immediately surrounded by small round cell elements, it may be embedded in a wide area of necrotic tissue, in which stains show very little structure; cases like this appear to correspond to rapidly growing forms of the disease. As in the other chronic infective granulomata, so here, in addition to the small round cells, macrocytes and giant cells are abundant. They are found in those cases where the fungus is making the least progress, or, in other words, in those cases in which the resistance of the tissue against the invading parasite is greater. Figs. 17, 21, and 22 show very striking

examples of phagocytosis; the phagocytes are similar to those which one of us found in enormous numbers in the case of *Aspergillus niger*.* Still even more striking are the huge phagocytic giant cells which may very often be found surrounding the smaller masses of the fungus; this appearance is represented in the drawing (fig. 22).

The hyphæ may ramify in the tissues for a considerable distance away from the main mass; this is seen, for instance, in uncleared specimens, where the hyphæ are represented by thick, solid, yellow trabeculæ. Sometimes phagocytes, as in fig. 17, or a large giant cell, indicate the place where a few ramifying filaments may be found upon careful examination. The direction of the ramifications may be determined by that of the connective tissue, trabeculæ, or vessels. We have already directed attention to the concentric vessel-like bodies scattered throughout the large nodule. Many of them enclose several hyphæ, and, from what we have seen in other specimens, it appears that the hyphæ penetrate the vessels, and ramify in them; they serve to guide the hyphæ, and no doubt the same is also effected by the long-coursing connective tissue trabeculæ. In the case of Aspergillar mycosis, previously alluded to, the penetration of a large vessel by the hyphæ was very well marked. It thus appears that the vessels may assist the extension of the fungus to no inconsiderable degree.

Nature and Significance of the Fungus.

CARTER, in his description of the fungus of Madura disease, evidently describes, though with very little detail, a parasite similar to that which we have just described; but he based his conclusions upon specimens which had only *partially* undergone metamorphosis, and in which, therefore, the hyphæ were clear. He concluded from these transition stages that the black masses, as well as the white bodies in the white variety, were of a similar fungoid character. None of our specimens show those transition stages, and, unless we had resorted to the clearing process, it would have been very difficult to demonstrate their fungoid character. It is most probable that the hyphæ are originally clear, and that subsequently pigmentation and metamorphosis occurs. Recognizing the probable fungoid nature of the black masses, CARTER looked upon them as corresponding to the *sclerotia* of the higher fungi. It must be remembered, however, that the hyphæ ramifying throughout the tissues undergo this extraordinary metamorphosis; the process is not limited to a particular portion of the fungus, nor is it connected in any way whatever—at least, in so far as we have been able to judge by the examination of many hundreds of sections—with a process of fructification. As far as we have observed, the interstitial material between the hyphæ is formed, to a slight extent, by the remains of the animal tissue in the immediate vicinity of the hyphæ, and the hyphæ themselves become obscured by an opaque golden-brown pigment; it is not a question of the thickness and browning of the

* R. BOYCE, "On Aspergillar Pneumono-Mycosis." 'Journal of Pathology and Bacteriology,' No. 2, 1892.

walls of the hyphæ, or of their forming a dense pseudo-parenchyma. The phenomenon appears to us to be an end or involution phase, rather than a transition phase. We are aware that, amongst the fungi, more than one *sclerotioid* process is described; but whether anything analogous to the above occurs we are unable, from want of experience, to state. It appears, however, to be a fact that parasitic sclerotia do occur, in which remains of the tissue of the plant host may be seen. DE BARY* mentions that CORDA† points this out in the case of *Peziza sclerotiorum*, but we have not been able to find where he makes the statement in his atlas. DE BARY himself figures *Sclerotinia Fuckeliana*, containing the cell *débris* of its vine-leaf host. In many respects the black masses behave like the sclerotia. Thus, if the sclerotium of of *Claviceps purpurea* is boiled for a short time in caustic potash, and then placed in water, there is the same streaming out of dark brown colouring matter. Both are readily cleared by *Eau de Javelle*. Nitric, hydrochloric, and sulphuric acids cause reddening of the black particles; the same effect is produced, not only upon the sclerotium of *Claviceps*, but also upon those of *Nectria*, *Peziza sclerotiorum*, and *Rhizomorpha*. As none of these are, however, delicate reactions, we lay no stress upon them. The black particles are very resistant to strong reagents; thus, as has been stated, they may be boiled in concentrated caustic potash for a considerable time, undergoing thereby only a slight change in colour. Sulphuric acid causes their disintegration, but they resist the action of nitric and hydrochloric acids for a considerable time; nitric acid produces some effervescence. Fig. 24 shows a small piece of the section fig. 4 which has been treated with hydrochloric acid, and then with potassic ferrocyanide; there is a marked green coloration of the *peripheral* tufts. We presume the green is due to the combination of a Prussian blue reaction with the natural brown-yellow colour of the fungus, and that it indicates the presence of iron. When the section is first cleared and then tested for the presence of iron, it is again observed that the peripheral tufts acquire a faint *blue* tinge, and, when a portion is highly magnified, numerous blue pigmented bodies, similar to those in fig. 25, are seen; they correspond to small collections of brown pigment which are nearly always to be met with in the disintegrating animal tissue surrounding the hyphæ; the iron in the colouring matter is probably for the most part of vascular origin. Thus a micro-chemical examination of the dark masses furnishes good evidence of the presence of iron, and this exactly accords with the chemical analysis. Chemical analyses have been made both by BRISTOWE and by LEWIS and CUNNINGHAM, and have revealed the presence of iron. BRISTOWE states that a small quantity of ash was left after combustion, and that it contained a little oxide of iron, but much less than a similar quantity of altered blood would have contained after combustion. According to LEWIS and CUNNINGHAM, the ash is of a red colour, owing to the presence of oxide of iron, and this fact points to an origin identical with the other pigmentary

* DE BARY, 'Comparative Morphology and Biology of the Fungi.' Oxford, 1887.

† CORDA, 'Icones Fungorum.'

deposits in animal tissues. Spectroscopic examination of the colouring matter of the brown masses does not any way favour a blood origin. We have ourselves incinerated thick pieces of the large black mass. The dried particles burn with a luminous flame. There is a smell of "burnt feathers." Heated on the platinum capsule, there is no sputtering whatever, and a residue is left white in the centre but brown at the periphery; the latter contains iron, giving the Prussian blue test, so that this peripheral distribution of the iron quite accords with the above micro-chemical observations. The iron is probably derived from the tissues, and not from the fungus. Whether the yellow pigmentary substance is of a resinous nature, and similar to that produced by certain species of fungi, it would be difficult to say. The dried particle burns readily; it is only very slightly cleared by boiling in carbon bisulphide, benzole, xylol, chloroform, and ether, and the special cupric acetate test for resinous substances fails, there is also no reaction with ferric chloride.

It is clear that, in the "sclerotioid" phase of the fungus, we have to deal with a process taking place in the living tissues which appears to be quite unique, and all the evidence is in favour of the view that the yellow colouring matter is furnished by the fungus.

One specimen of the black variety, from which figs. 11, 12, and 17 are made, shows unmistakeably that the tissue is offering considerable resistance to the invasion of the parasite, and that in consequence the latter is altered in appearance. The tufts are radiate, but, instead of the ends of the hyphæ being loosely arranged, they are grouped into a dense palisade, as seen in fig. 17A; some are thickened and club-like, and they are invested by either phagocytes, fig. 17, or giant cells, fig. 22. The interior of the tuft is occupied by an irregular large cell pseudo-parenchyma, which is slightly pigmented, and between this and the above-mentioned palisade, the hyphæ much reduced in size form a deeply pigmented dense zone, figs. 12 and 17A. This type is interesting to compare with those specimens in which both the tufts are very numerous, and the hyphæ run in the one direction, whilst at the same time the destruction of the tissue is great.

As regards the relationship of this fungus to Madura disease, we think that the anatomical evidence brought forward shows that it is pathogenic. The fungus is scattered in large quantities throughout the tissues, and there appear around it areas of necrosis, of granulation tissue, of phagocytes and of phagocytic giant cells. The process *spreads*, and fresh areas of the foot, of the leg, or even of the thigh become invaded by the black particles. But it is very extraordinary that in our six specimens we have only met with one in which the peculiar pigmentary metamorphosis was not marked. Owing to the rapid metamorphosis it seems to us that the cultivation of the fungus from the particles will be difficult. Two doubtful cases have been recorded by CARTER, and the other by LEWIS and CUNNINGHAM, of the occurrence of the black and white particles in the same specimen. We likewise possess in our college a specimen in which both black and white particles were

found; but, whether owing to the bad preservation or not, we have always failed in this specimen to demonstrate the tissue changes which could be attributable to the growth of the fungus; there appears in this specimen no relationship between the black fungus and the tissue, and it is very possible that the black particles may have fallen into the cavities of the foot, from being placed in the same vessel along with specimens of the black. On the other hand, the presence in the same foot of two distinct parasites, or of one parasite succeeding the other, is far from being an impossibility.

The case of BASSINI, mentioned at the commencement of this paper, is interesting in connection with the pathogenicity of this disease. In this case the patient pricked his foot in an Ox stall. The wound healed, but a tumour gradually formed which broke upon the surface, and, between the seventh and eighth month after the injury, prevented walking. The tumour, which had reached the size of a pomegranate, was removed; it was found to be pervaded by dark brown or black particles varying in size from a pin's head to a hazel nut, and there were numerous fistulæ from which the black particles protruded. As previously mentioned, treatment with caustic potash revealed a septate mycelial fungus. BASSINI did not succeed in cultivating it.

The White Variety of Madura Disease.

Of this variety we have examined sixteen cases, and we have found that in those specimens where the characteristic roe-like bodies are present the structure is uniform. The appearance of a section of a particle under the low power, is well seen in fig. 14. It is extremely characteristic, and it appears to us difficult to conceive how it could have been confounded with the black variety. The particle consists of an aggregation of deeply staining "reniform" bodies and of a radiate external zone. In this section the particle is seen to be surrounded by granulation tissue, but it may be free in large numbers in the sinuses and be discharged with the pus as the fish-roe bodies. Usually about the size of a pin's head they may yet be often found of the size of a pea; they are most frequently nodular upon the surface. They are soft and friable, and possess a very light brown or, as it is termed, "ochroid tint," but they are never yellow, as in *Actinomyces.*

A considerable number of the observers who have examined these bodies have failed to find any vegetable structure, whilst in the case of those who have recognised a fungus, the descriptions have, we venture to think, been rendered very misleading, owing to confusing the two varieties.

The particles are, to a great extent, concretions of caseous and probably phosphatic materials upon a nucleus which possesses traces of a fungus. To examine the fungus it is, therefore, necessary to remove as much as possible the foreign materials. To do this we treat either the free particles or the sections of the particles in the tissue with the various fat solvents. Thus the particles are *heated* in absolute alcohol, ether,

carbon bisulphide, chloroform, xylol, or benzole, for from a few minutes to half an hour or longer. Particles treated in this manner are clearer and take up the logwood stain much more readily than untreated specimens; yet, notwithstanding, their bulk cannot be said to be much diminished; a quite similar result may be observed in the case of caseous material. Definite acicular crystals are often met with after treatment with the above-mentioned fat solvents, so that they are probably not fatty. After these reagents we very frequently employ fuming hydrochloric acid; this renders the outlines of the fungus sharp, but there is only a slight solvent action. In contrast to the black variety, strong caustic potash, or *Eau de Javelle*, cannot be used, as those reagents soon cause the complete destruction of the particles. This shows that the vegetable structure cannot be very resisting. For embedding sections we always employ the paraffin ether method; celloidin gives most misleading results, as may be readily seen by comparing side by side sections prepared in the two ways.

The dark kidney-like masses seen in fig. 14 are most probably fungoid. They stain very deeply with most aniline and logwood stains, but it is exceedingly difficult, notwithstanding, to make out a definite vegetable structure. They appear for the most part to be granular, the granules being small and very densely packed. We have seen, however, in two specimens, unmistakeable evidence of a very delicate branched hyphal network. One of these cases has been recently described by Dr. HEWLETT,* and, from the appearance, he concluded that it was a fungus similar to *Actinomyces.* The other case was one examined by Dr. KANTHACK. We have ourselves examined an early and well preserved foot which was sent to us direct from Bombay. There are no particles visible to the naked eye, but the sections show great leucocytic massing, and the commencement of the formation of minute abscesses and sinuses. Here and there in the leucocytic centres, small granular reniform or annular masses may be seen which stain only slightly with gentian violet; here, occasionally, however, very short and extremely delicate deeply stained filaments may be seen in the apparently granular masses but there is no radiation, nor are there clubs to suggest a relationship with *Actinomyces.* Fig. 15 shows an appearance also very commonly met with in the particles, namely, the formation of a *deeply staining* irregular fringe around the reniform bodies. Examined with the high power, the fringe may be seen to consist of stunted, rather thick hyphæ; sometimes there is a very large number of the stunted projections, and double staining with HOFFMANN'S green and eosine may give very striking pictures, the fringe staining green, and the reniform masses a reddish-purple. The walls of these dwarfed club-like processes may be greatly thickened, and they closely resemble many of the clubs to be seen in the ray fungus. We have not in our specimens, however, succeeded in tracing the hyphal processes into the central granular masses. Exceptionally the clubs are long. A third characteristic of the white particle is the radiate zone which

* HEWLETT, 'Lancet,' 1892. Dr. HEWLETT has very kindly shown us his preparations and given us material, and we agree with his description.

surrounds the reniform masses. The appearance of this radiation is extremely well shown in the very distinct high power photo., fig. 16. Exceedingly delicate straight brush-like processes radiate out for a considerable distance from the central deeply staining mass, whilst between the radii are numerous compressed leucocytes. In a few cases the slender radii are thicker and more club-like, in others they are represented by thick radiate refractive masses in which no structure can be made out.

Such then are the leading features which we believe can be seen in the roe-like particles; some of them favour a relationship with *Actinomyces*, but from others we cannot in our present state of knowledge draw any conclusions. The deep radiation is a most remarkable phenomenon, and one which has greatly struck both CARTER and LEWIS and CUNNINGHAM; these observers looked upon them as consisting mostly of fat crystals, and although we also think that fat crystals may greatly increase the radiate appearance, yet we have found the radiate zone, as in figs. 14 and 16, after prolonged subjection of the sections to the fat solvents mentioned above. Coupled with these curious facts we have found others equally hard to interpret. Thus, in addition to the opaque ochroid roe-like bodies, we have met with others, transparent and gelatinous, which, when pressed under a cover slip, exhibited a radiate structureless and refractive peripheral zone; yet, from microchemical testing, we could draw no conclusions as to their nature. Similarly, when we read the records of CARTER and LEWIS and CUNNINGHAM, we find that they record cases in which the specimens contained innumerable cayenne pepper-like granules.

Comment upon these various anomalies would be out of place, and therefore, in spite of the fact that although we have examined comparatively a very large number of specimens, we think it would be rash to state that the white particles are always Actinomycotic, and may not in certain cases represent some other parasite.

Conclusion.

The object of this paper has been to show that in the Madura foot disease the white and black particles are of different nature, and to set forth the means whereby these differences can be displayed.

ADDENDUM.

Since this paper was written we have had the opportunity of examining a very large number of specimens of Madura disease, including fifteen examples of the black variety. For these we have to thank Professors GREENFIELD, of Edinburgh, and HAMILTON, of Aberdeen, and Dr. ROLLESTON, of St. George's Hospital, but especially Brigade-Surgeon Lieutenant-Colonel KEITH and Dr. BOCCARO, of Hyderabad, Sind. Dr. BOCCARO sent to us the MS. of an analysis of *one hundred* cases of Madura disease treated in the Hyderabad Hospital, and portion of this was published in the 'Lancet,'

September, 1893. *The vast majority of these cases appear to belong to the black variety;* and, with one or two exceptions, the specimens sent to us from Hyderabad, Sind, belong to the black variety. This points to a local distribution for both the black and the white variety. We may add, also, that we find considerable differences between the gross anatomical changes met with in the black and white varieties respectively. Dr. BOCCARO finds, in 17 of the 100 cases analysed by him, evidence of the pricks of an Acacia thorn (the "Babul"); in many of these cases the thorn was found. He also records a case of mycetoma between the vertebral column and the scapula. Dr. KEITH has kindly made for us very careful inquiries for the presence of Madura disease in cattle, and has been informed by the Veterinary Surgeon of its occurrence; in one case in the foot of a Camel. Dr. KEITH will, however, not pronounce a decided opinion till he has had an opportunity of examining a specimen for himself.

Drs. KEITH and BOCCARO have also inoculated several glycerine-agar tubes for us, but we have not succeeded in cultivating the black particles. We are, however, expecting more, and, one of us having left for India, we hope shortly to be able to transfer our test-tube experiments to the animal.—December 11th, 1893.

DESCRIPTION OF PLATES.

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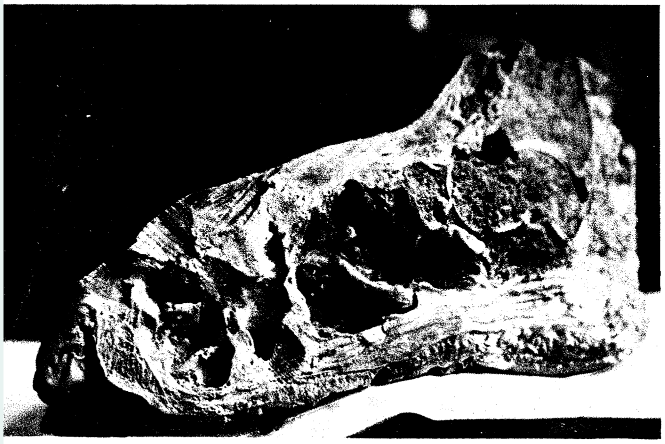

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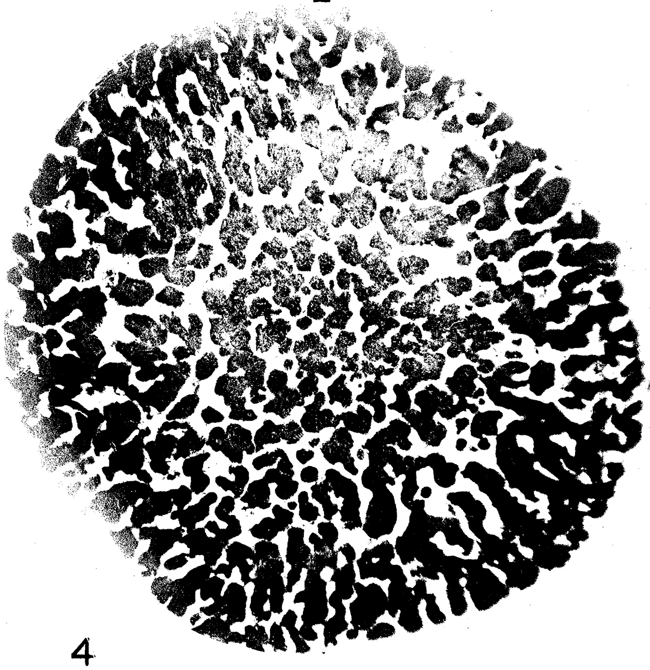

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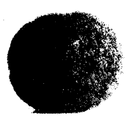

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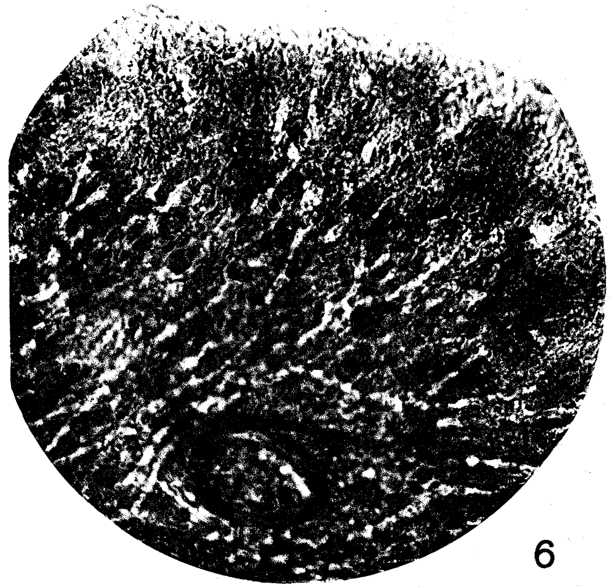

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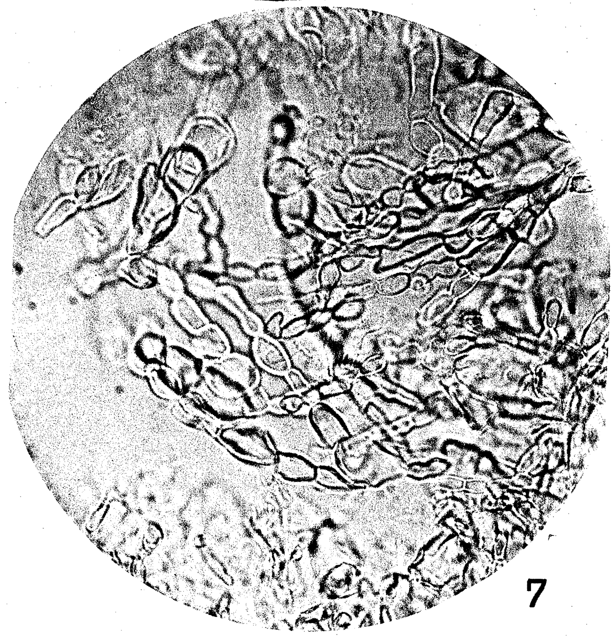

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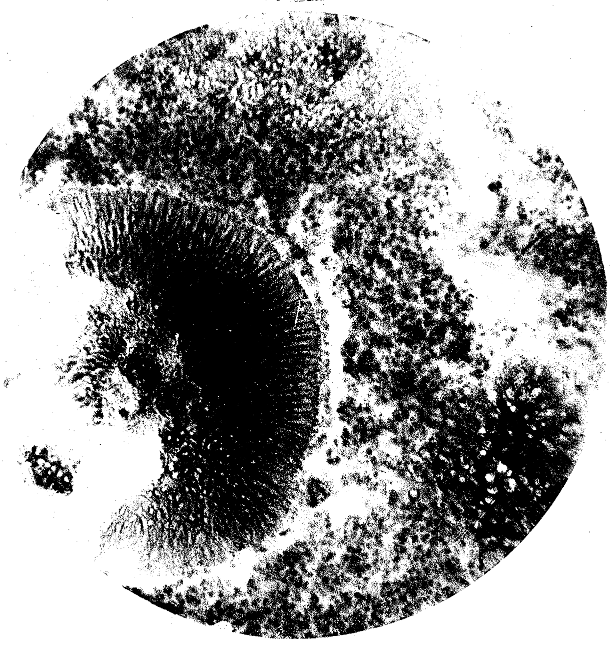

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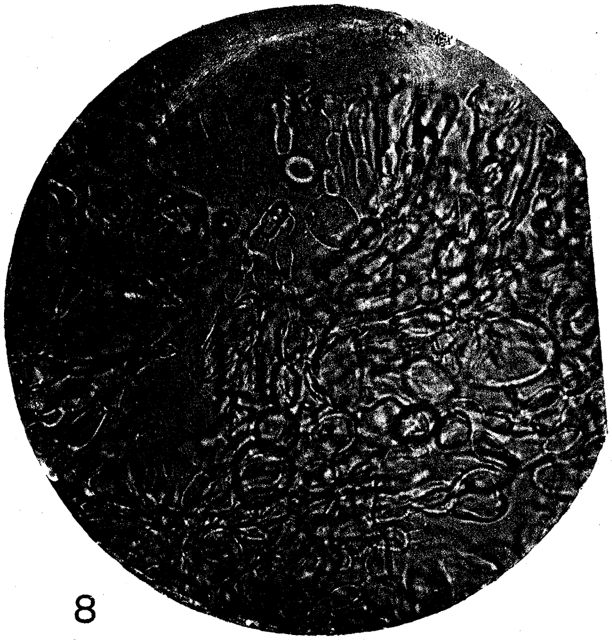
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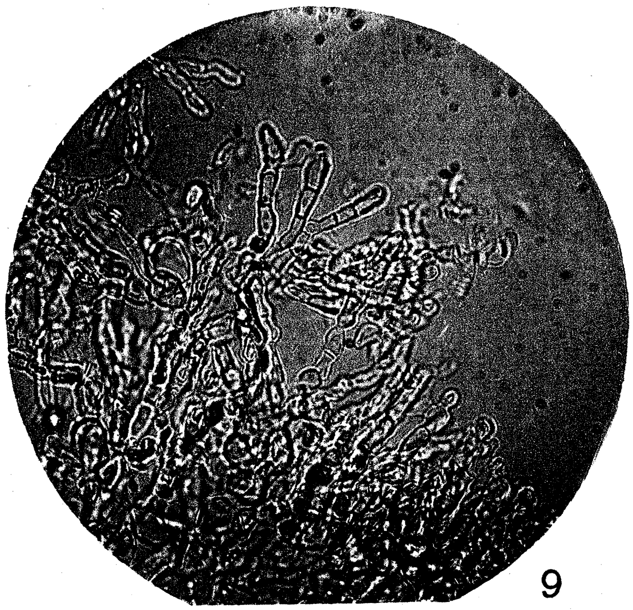
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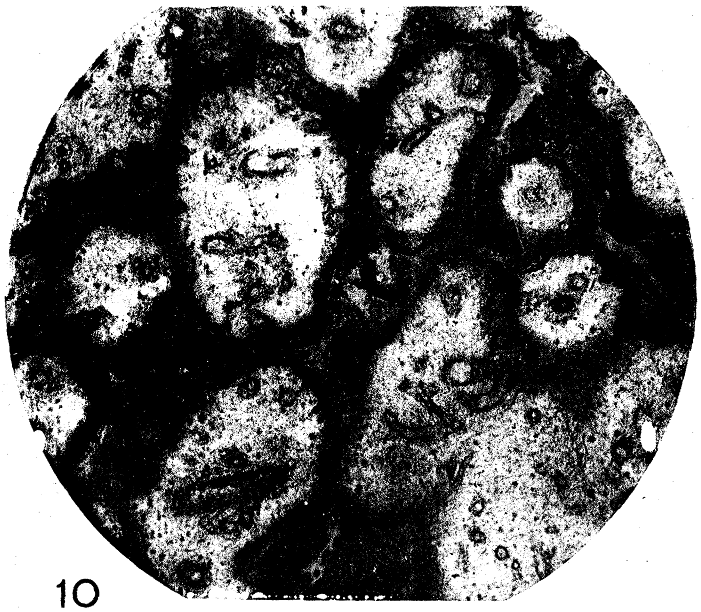
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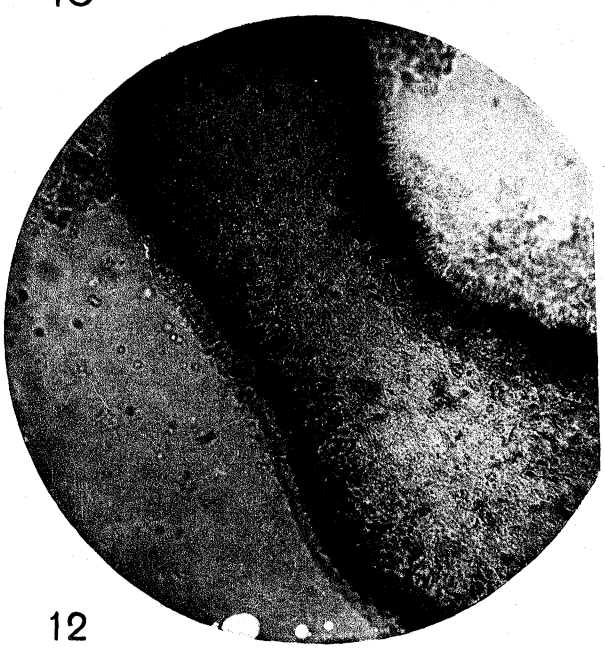
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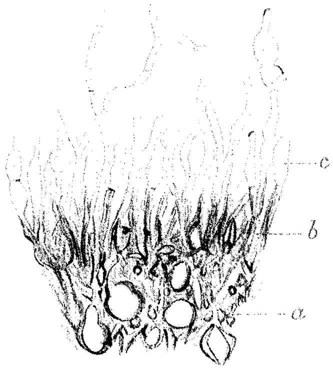

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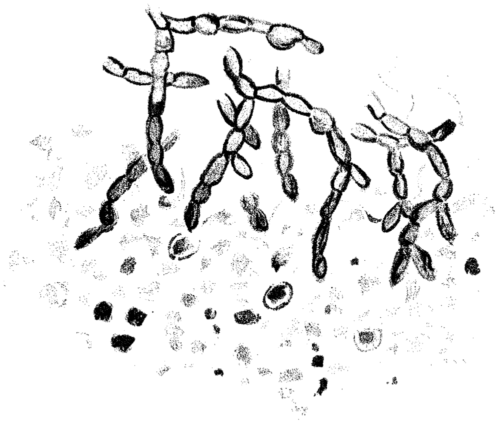
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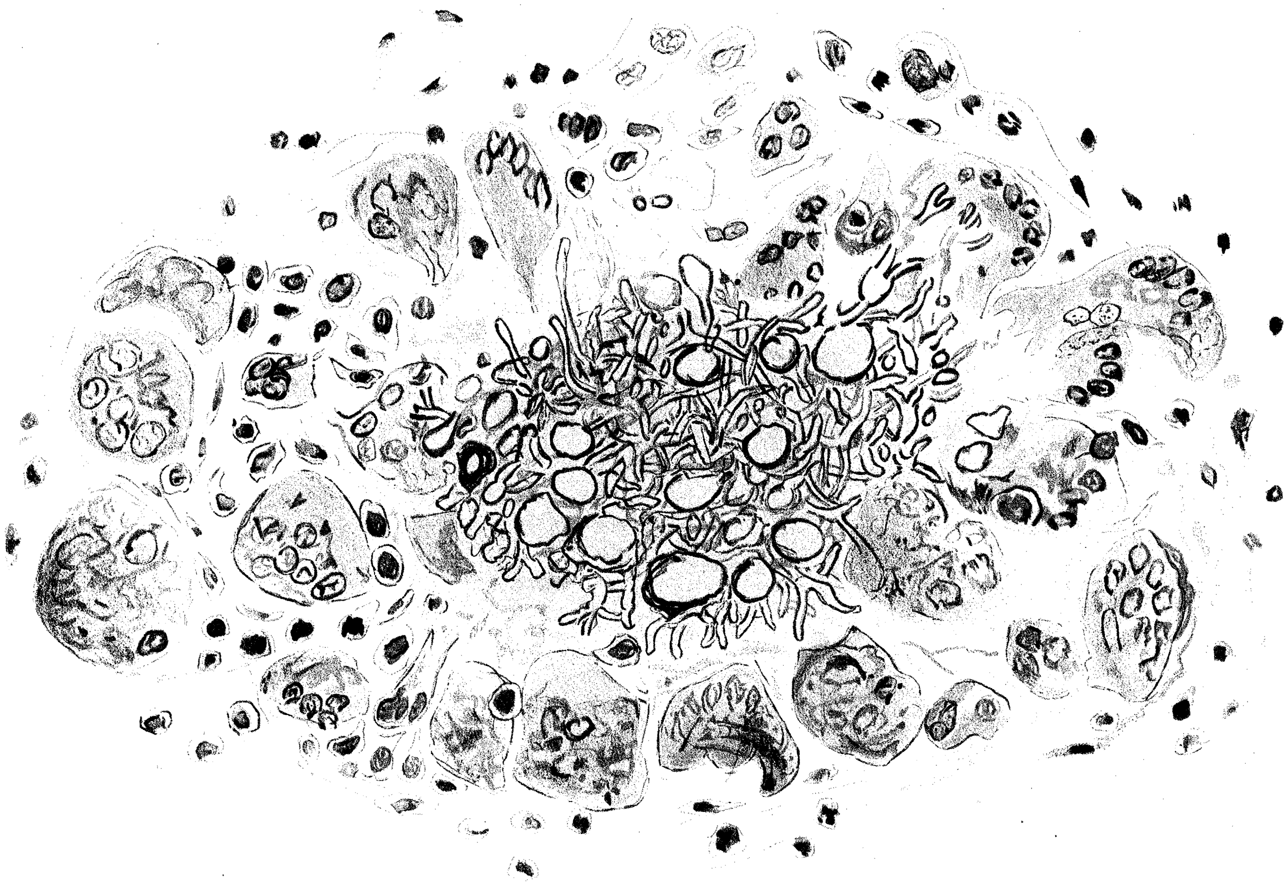
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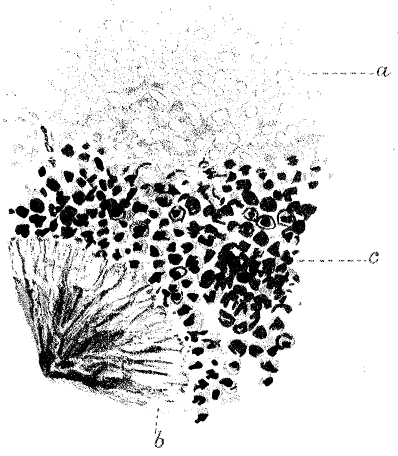

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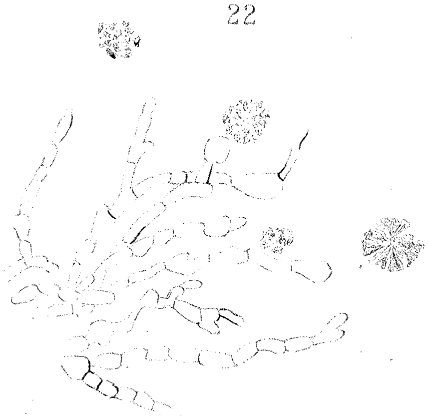
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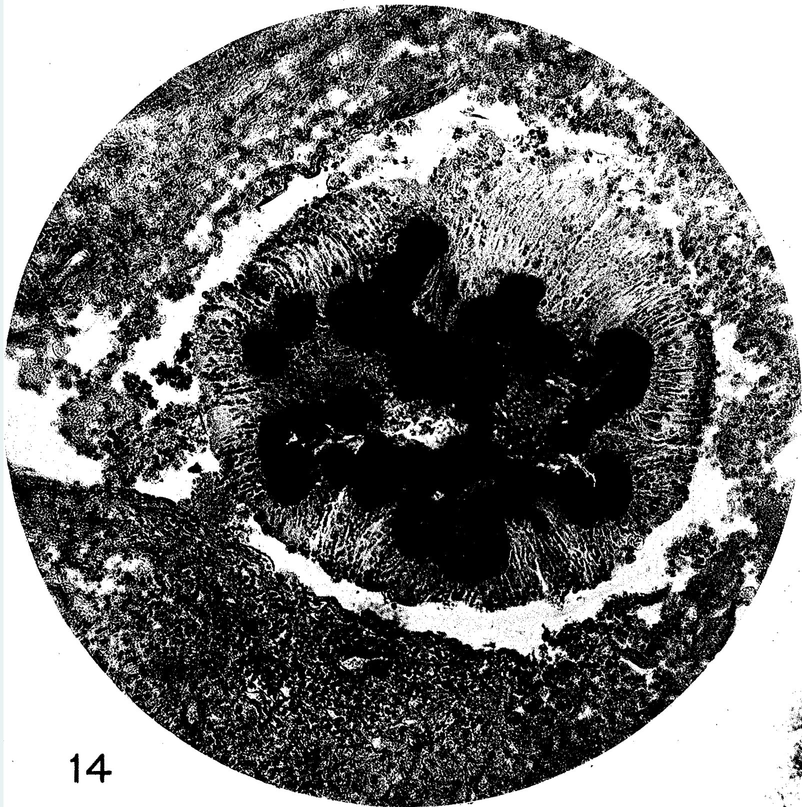

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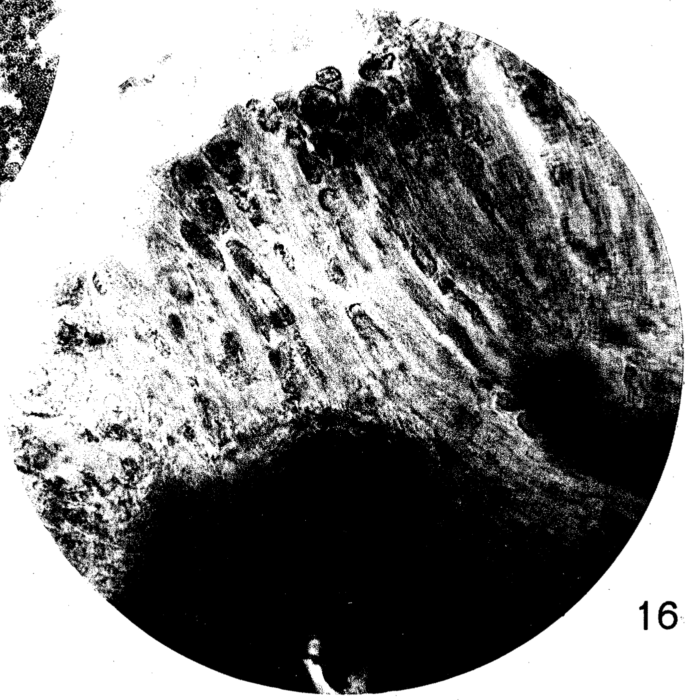

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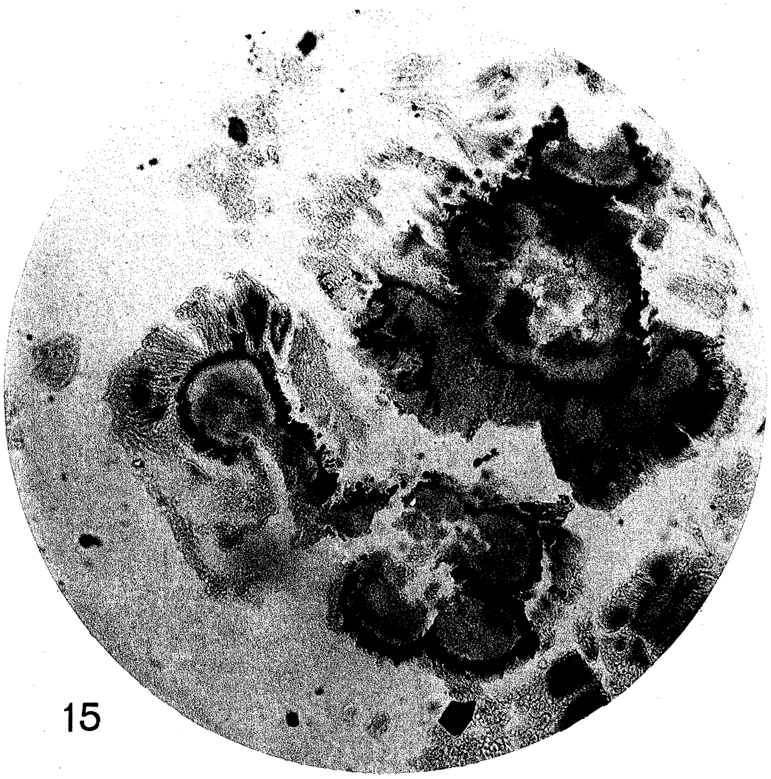

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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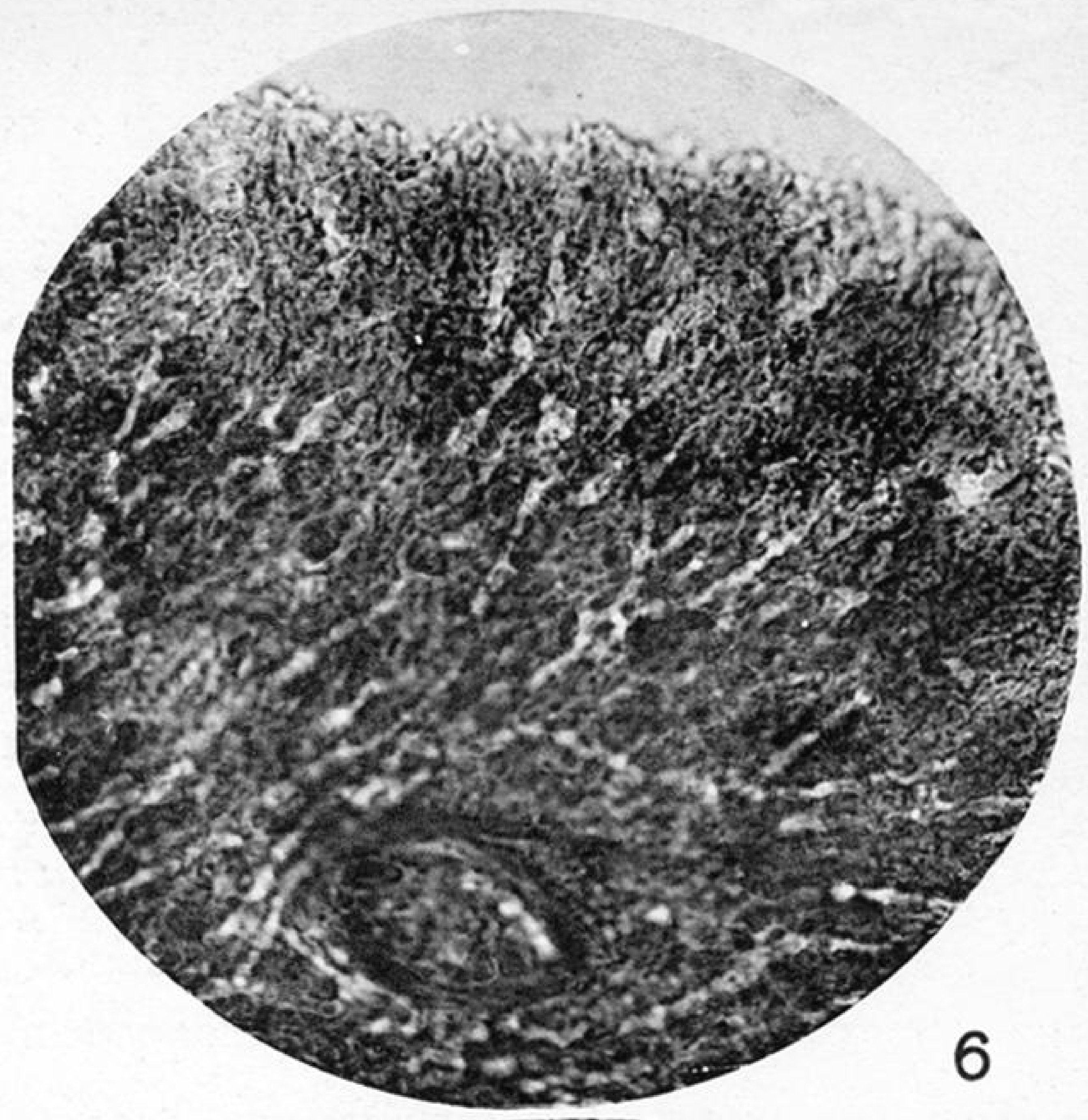

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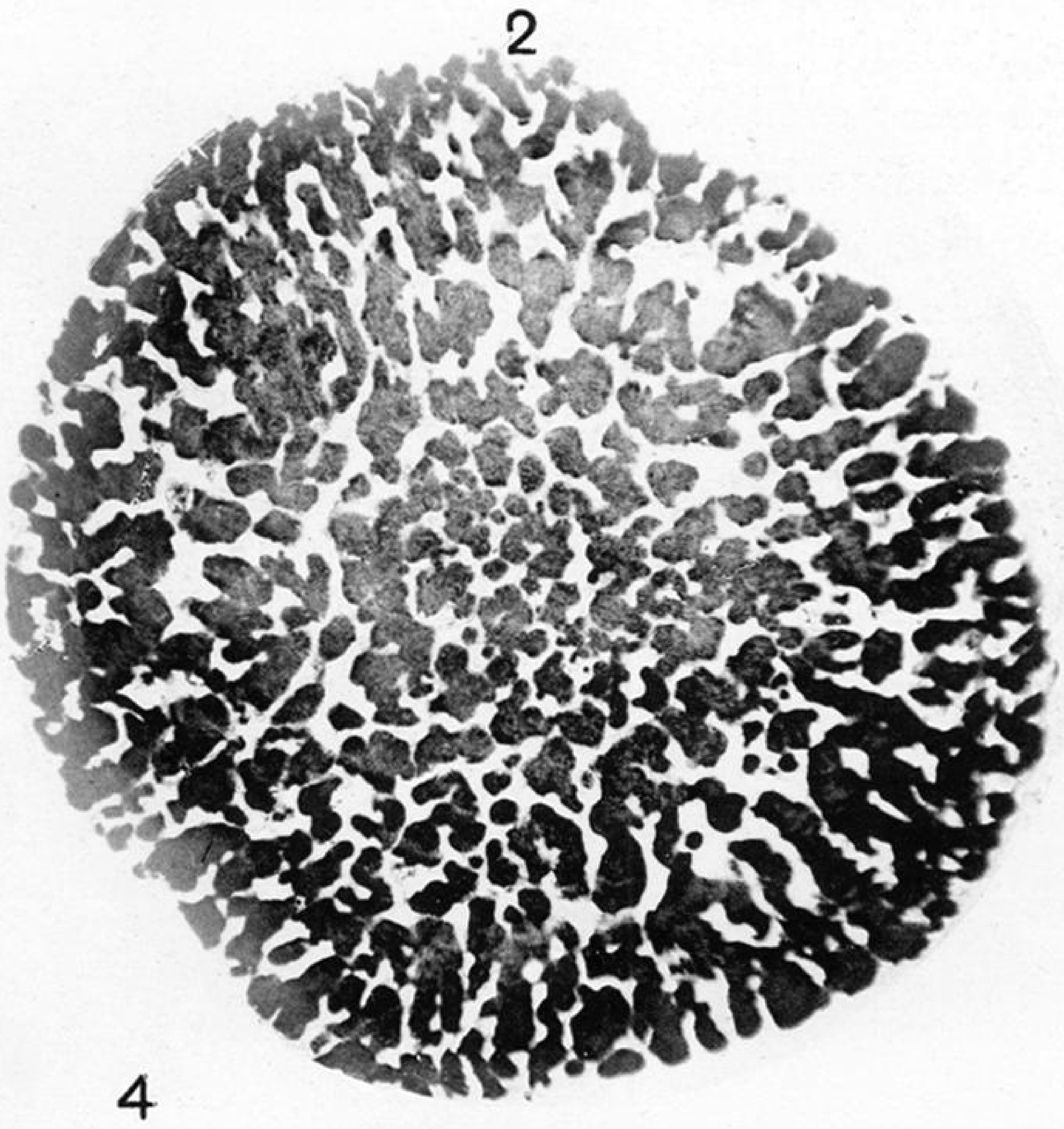

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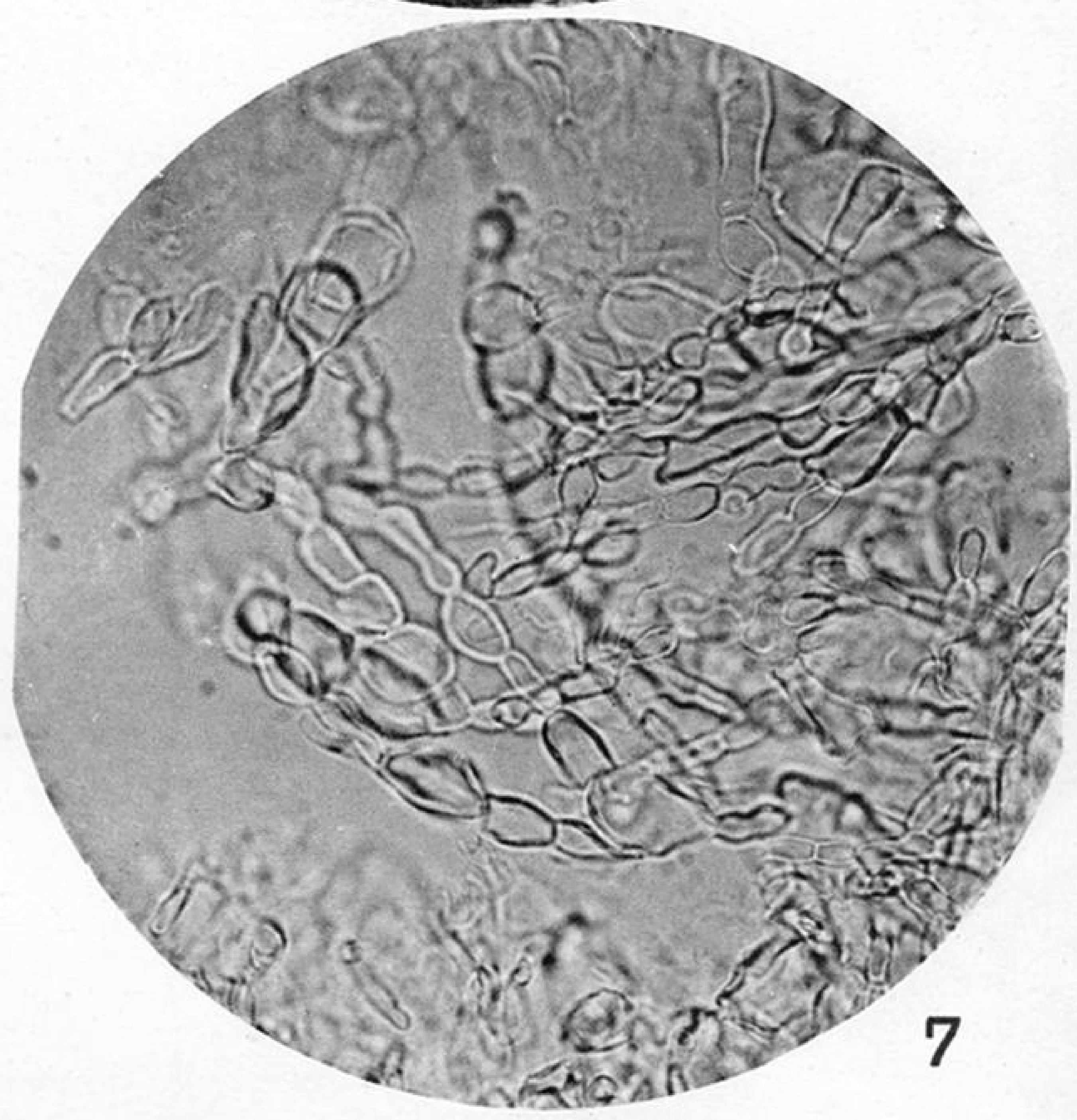

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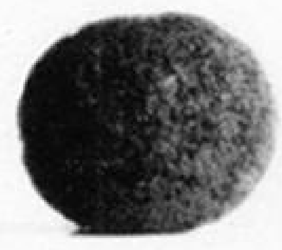

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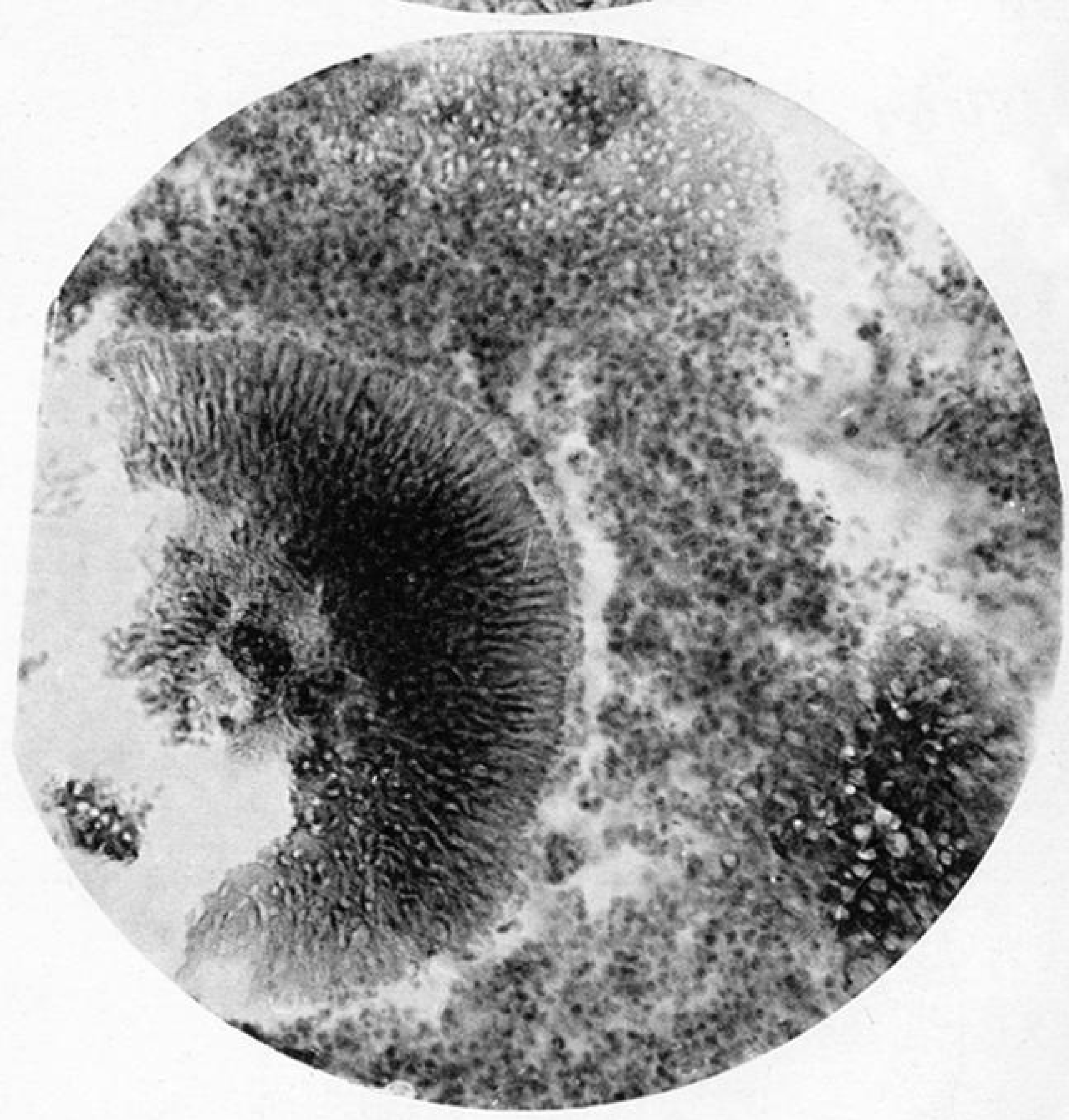

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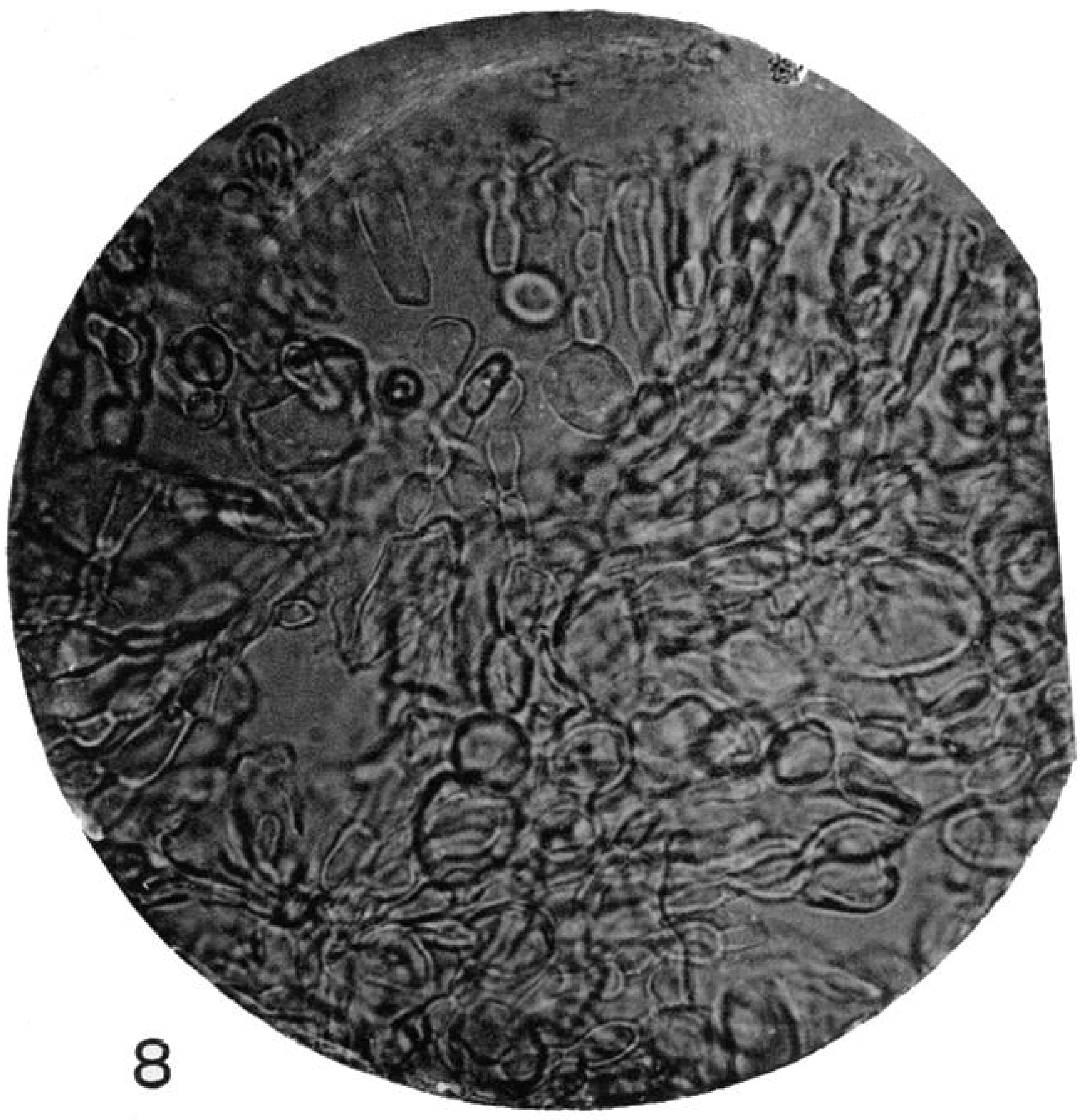

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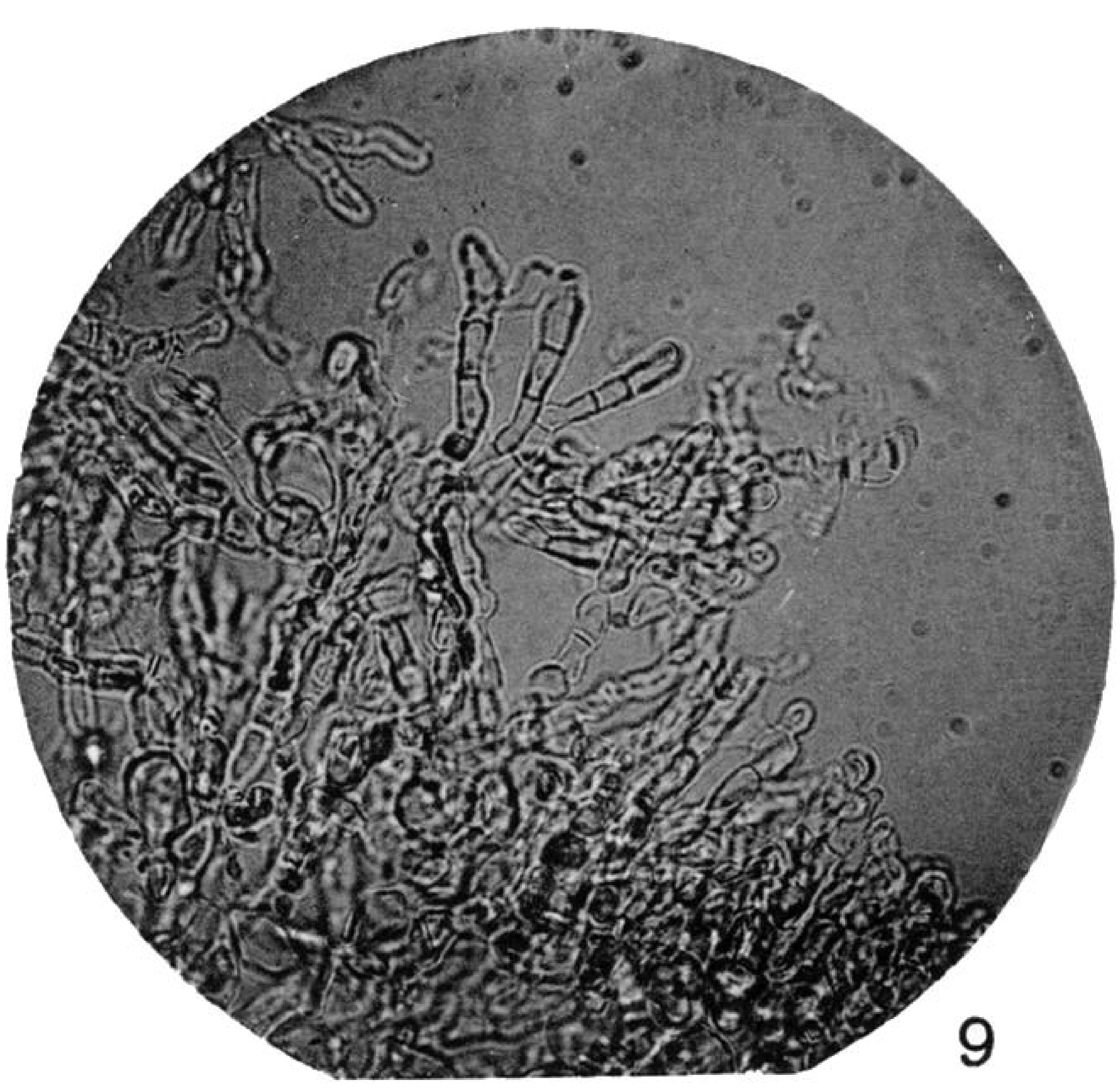

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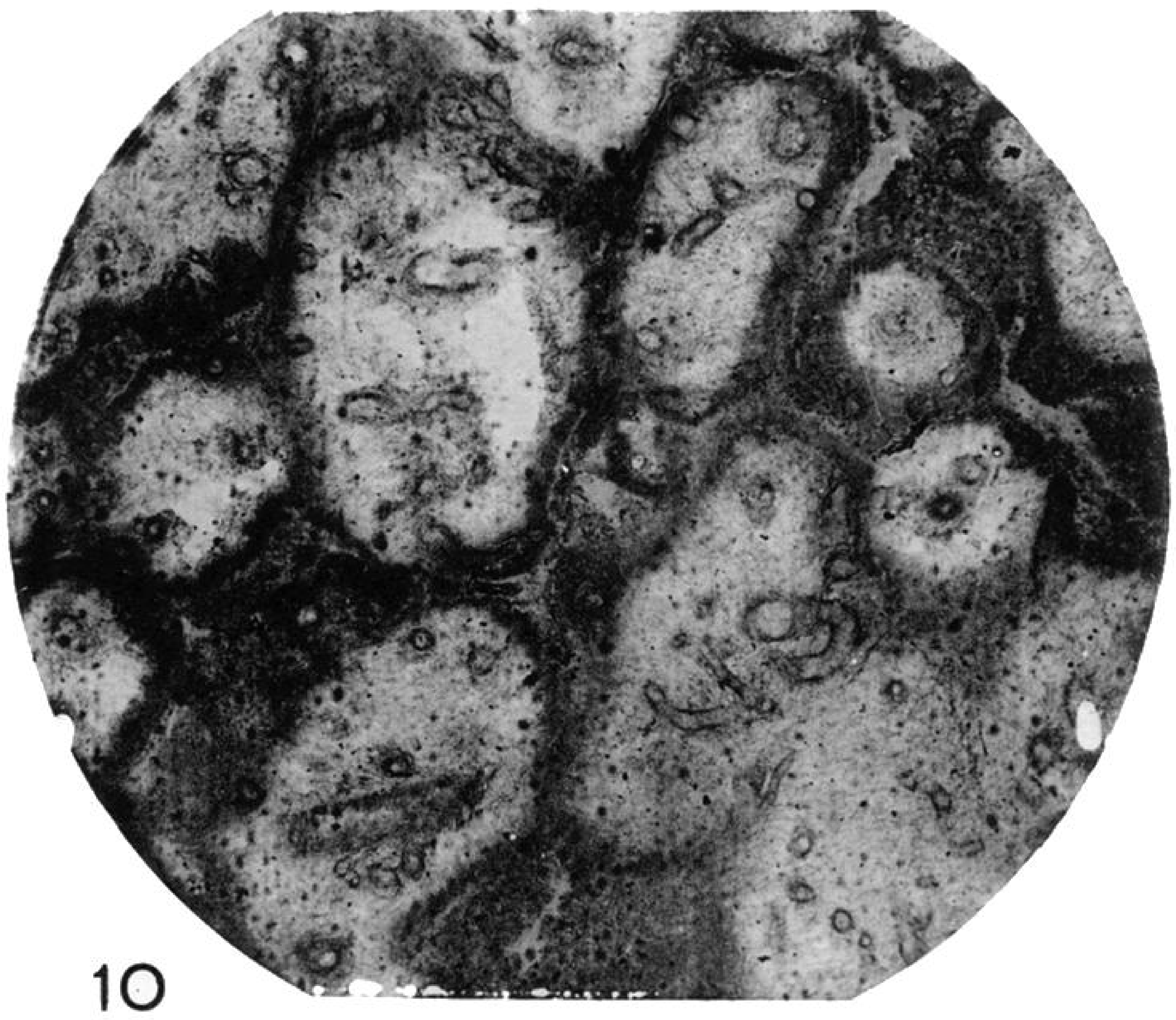

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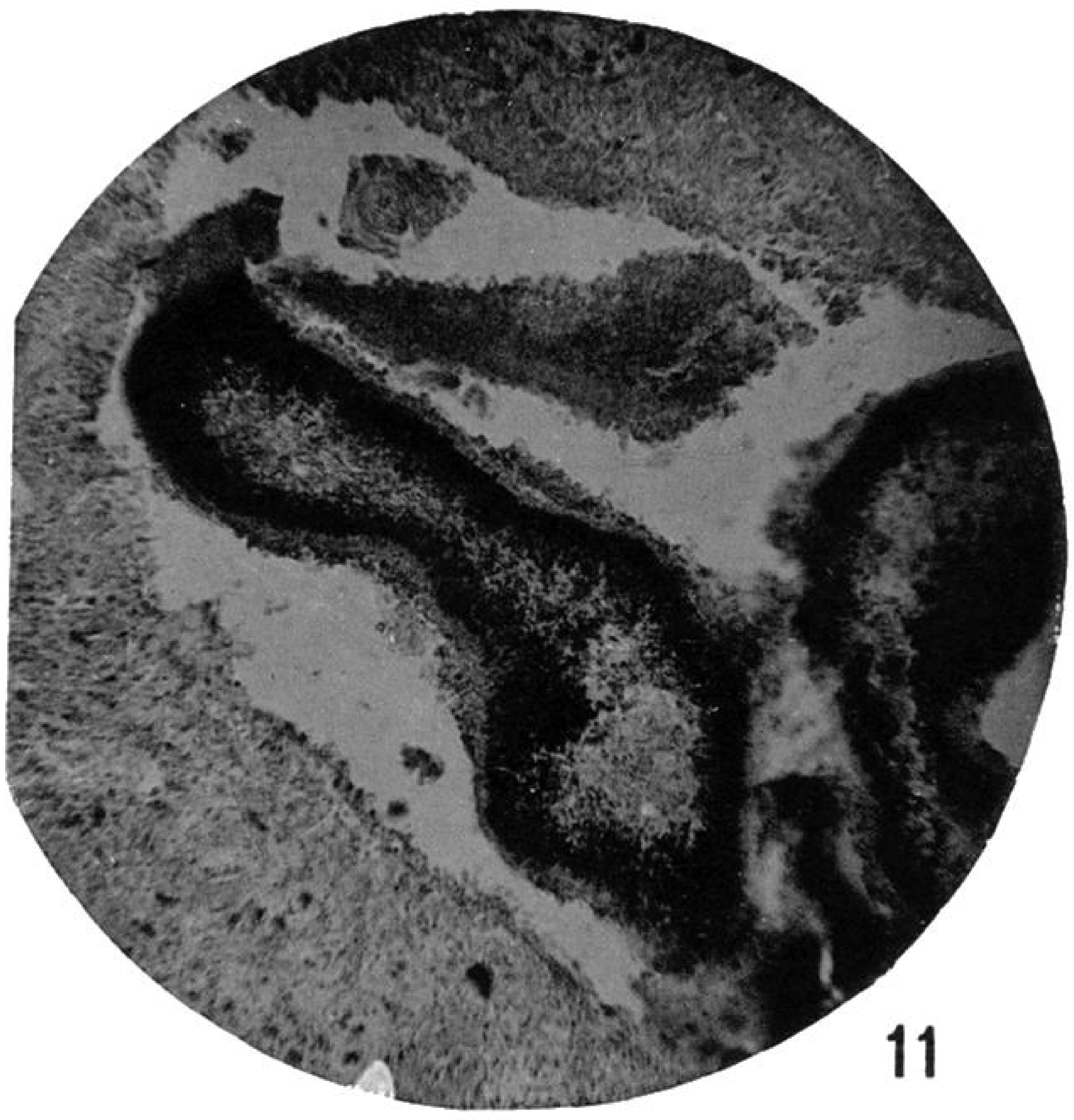

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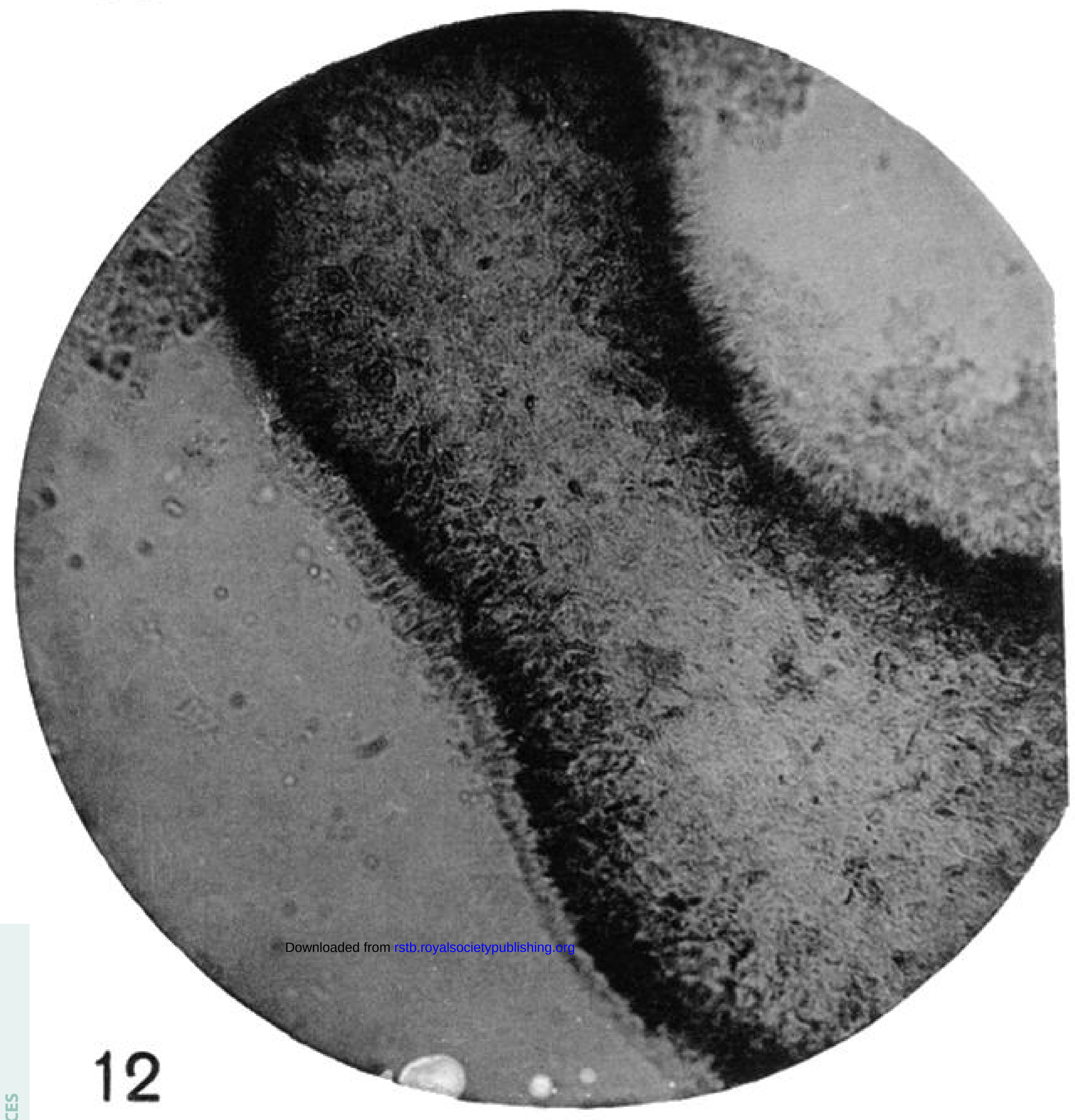

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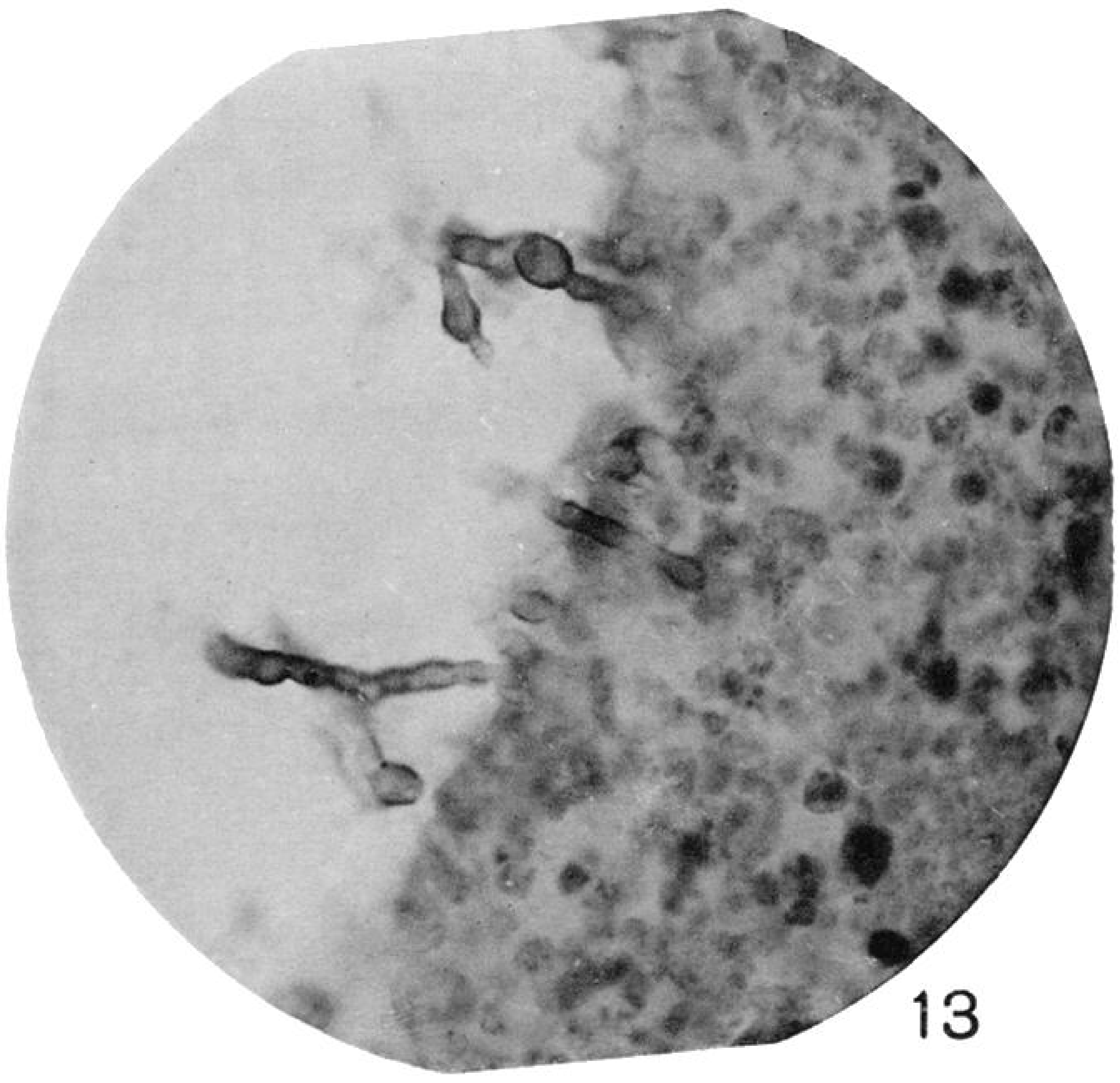

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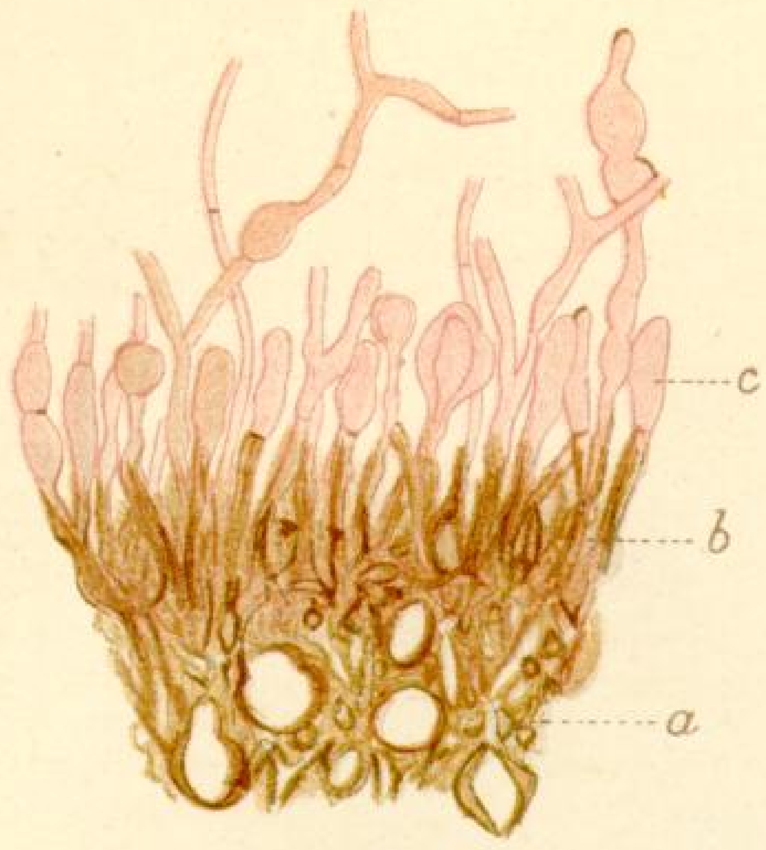

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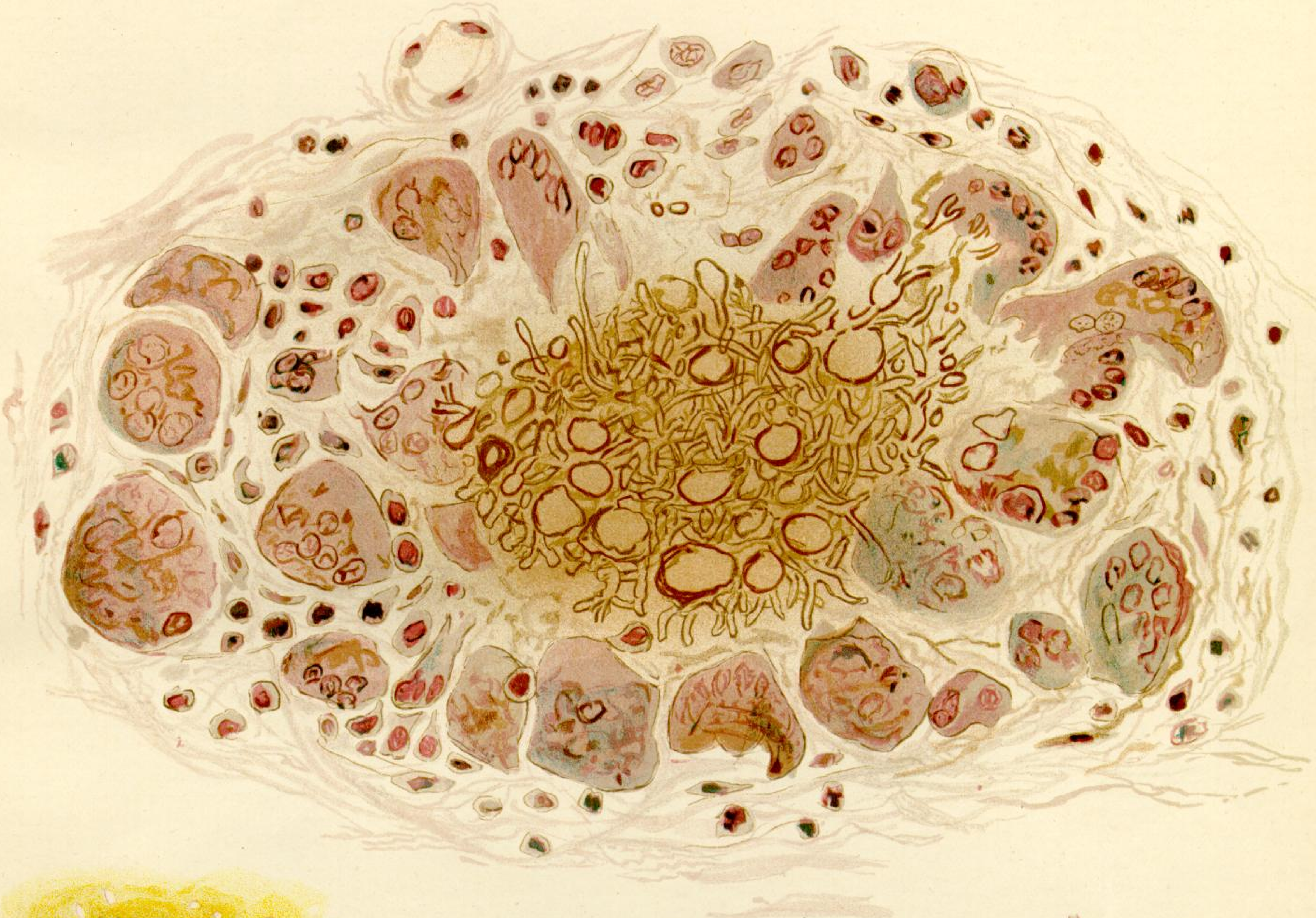

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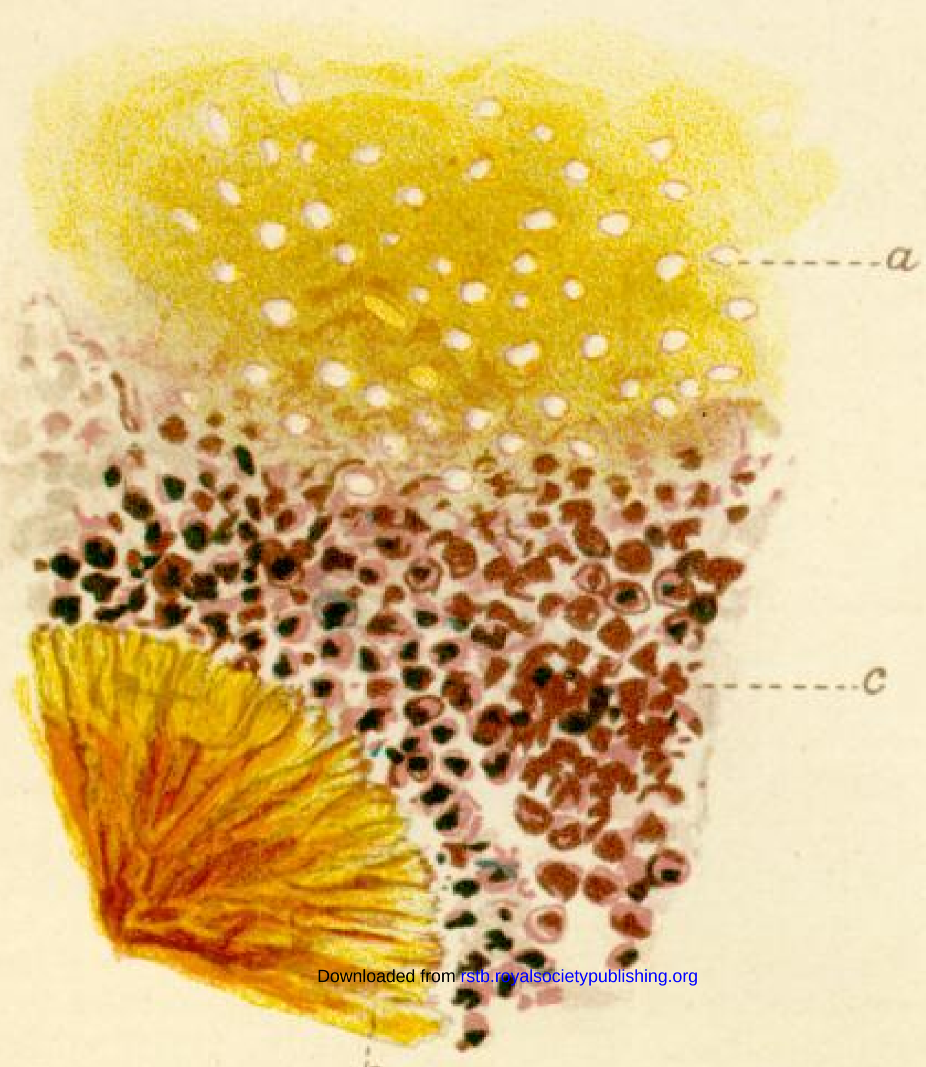

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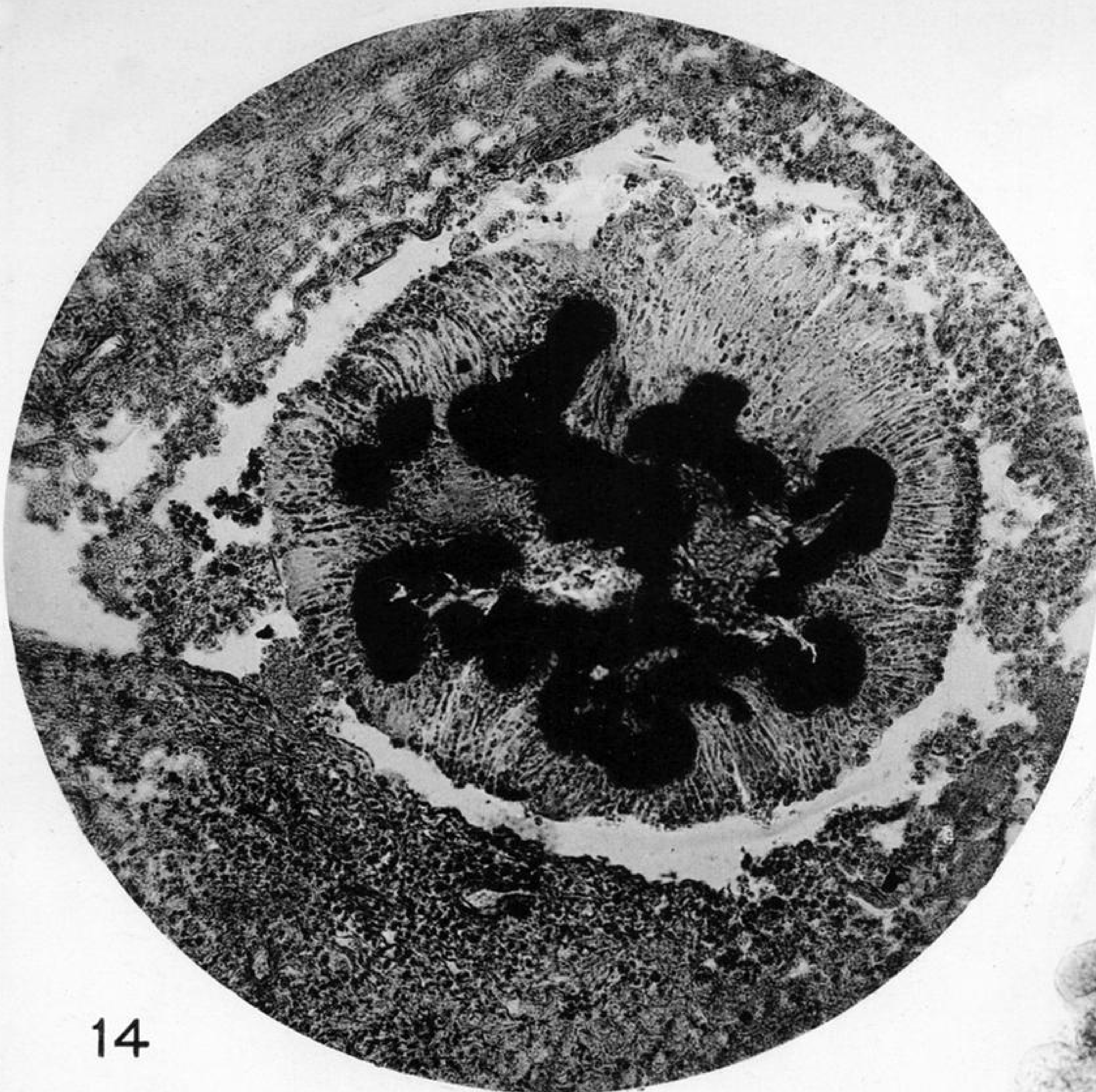

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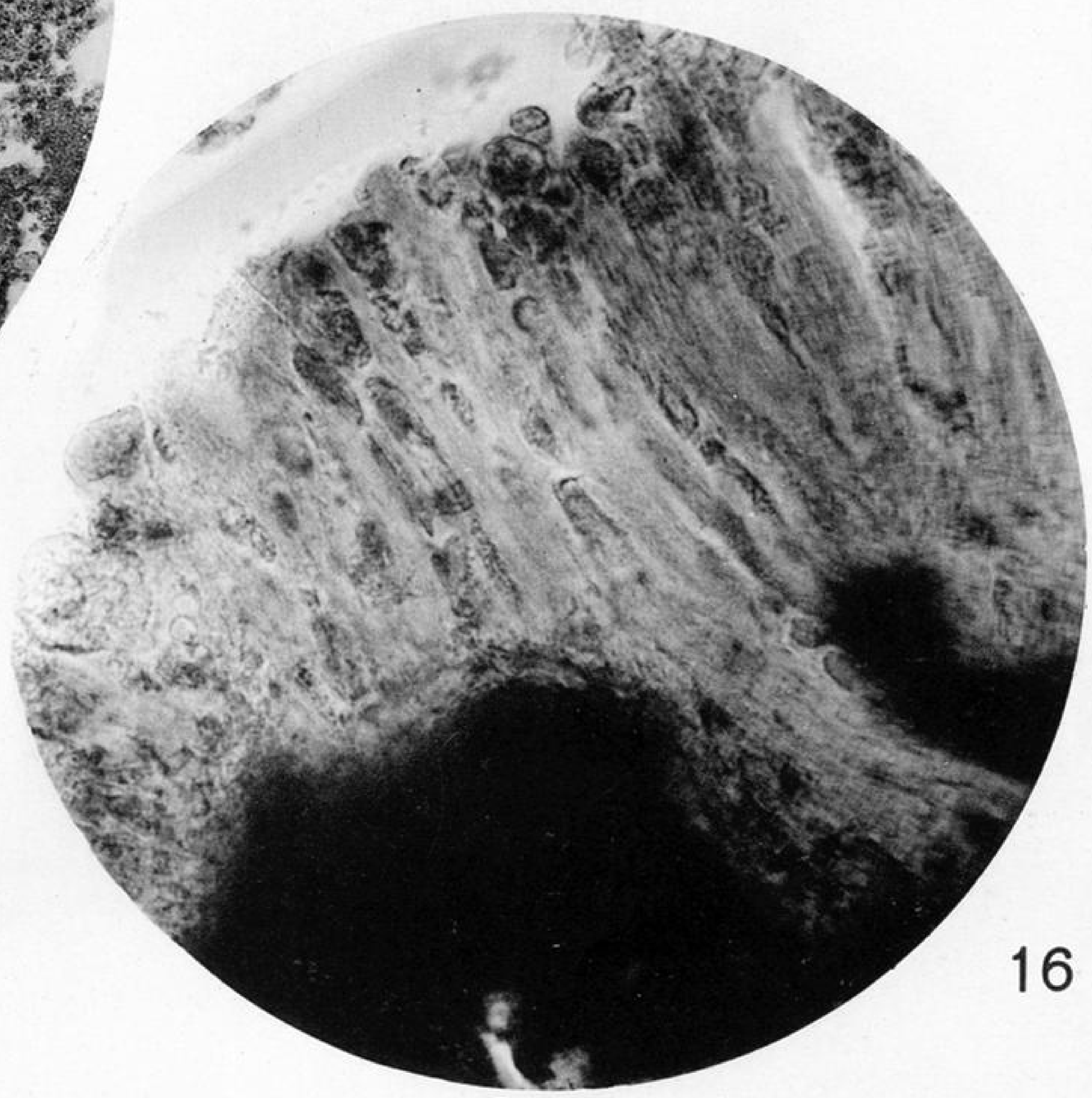

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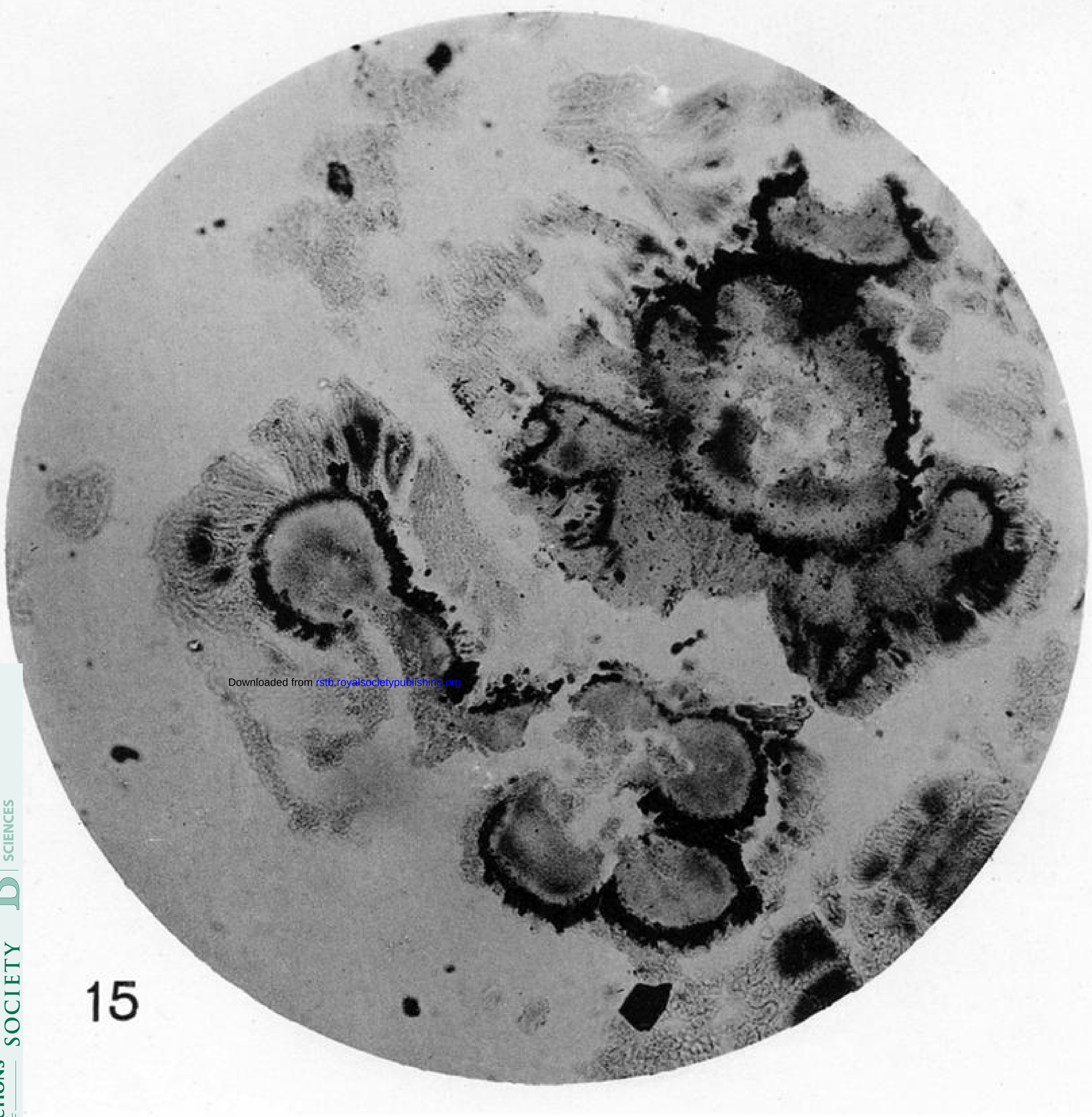

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